THE

BOTANICAL GAZETTE

EDITOR
JOHN MERLE COULTER

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ERRATA

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P. 86, line 7 from top, for *Oenothera*×*Onagra* read *Oenothera* § *Onagra*

P. 113, line 12 from top, for 235:1 read 255:1.

VOLUME LIX NUMBER 1

THE

BOTANICAL GAZETTE

JANUARY 1915

THE MORPHOLOGY OF ARAUCARIA BRASILIENSIS

III. FERTILIZATION, THE EMBRYO, AND THE SEED

L. Lancelot Burlingame

(WITH PLATES I–III)

Since the publication of the author's account of the male (3a) and female (3b) gametophytes of *Araucaria*, certain additional facts have been observed. They will be recorded here by way of preface to the following account of the embryo and seed.

In the paper on the male gametophyte the pollen was described as lodging on the ovuliferous scale and then growing over the surface to the micropyle, and a figure was given showing a number of pollen tubes. These pollen tubes were shown pursuing a more or less direct course to the micropyle. It was stated that they sometimes crossed from the upper surface of the scale on which they had germinated to the under surface of the scale above. Since that was written Eames has described the behavior of the pollen tubes of *Agathis* (8). The very interesting behavior of these tubes led me to re-examine those of *Araucaria*. It was found that while they do not, apparently, penetrate the tissues of scale and cone axis in the remarkable fashion characteristic of the sister genus, they do, nevertheless, branch much more profusely than had been supposed. It is often possible to separate a branching tube from the adjacent scales without breaking many of these branches. From this it appears that they are comparatively superficial. There is usually one main branch of the tube that goes more or less directly to the nucellus. From this numerous,

much smaller branches are given off. These branches run in all directions over the surfaces of the adjacent scales, so that when they are separated the mesh of tubes sometimes appears like a fine spider web. Numerous nuclei were distributed throughout the tubes, sometimes singly and sometimes in groups of a half-dozen or more. Aside from the body cell they all look much alike, so that it was not possible to identify the stalk and tube nuclei. The tube branches appear to grow independently of the tube nucleus or of any other nuclei. Branches were sometimes found in which no nuclei could be found at all. This was true of those of considerable length, as well as those that were just beginning to form. No cross-walls were observed in any part of the main tube or its branches. The main branch after entering the nucellus sometimes branches again (fig. 1). These branchings are very much less frequent than those outside. The available evidence indicates that only the one branch enters the nucellus.

In the majority of the tubes examined the body cell remains in the extra-nucellar part of the tube until after its division. Sperms were often observed in the tubes at the extreme tip of the nucellus (fig. 5), while very few body cells were observed within the nucellus. The mitosis of the body cell nucleus was not observed. Fig. 2 shows what appears to be the spindle lying between the two nuclei of a body cell. The nuclei are both in the resting condition and the spindle is surrounded by a delicate membrane. This membrane and its continued persistence after the daughter nuclei have reformed may be urged against this structure being really a spindle. It strongly suggests the remains of the nuclear membrane and thus suggests an intranuclear spindle. I have observed no other structures with which it could be identified, unless there may be some connection with the blepharoplast-like body referred to in a later paragraph as occurring in the cytoplasm of the male cells and the two-celled proembryo.

After the division of the body cell nucleus the two daughter nuclei do not usually separate for some time. In some cases they even enter the archegonium still closely associated. The division of the cytoplasm of the body cell follows considerably later. In some cases no distinct division has occurrred when the two male

cells have reached the archegonium. The body cell is commonly surrounded by a very faint membrane (figs. 3 and 6). In some cases this membrane is either absent or so faintly marked as to be exceedingly difficult of observation. The line of demarcation between the two male cells is in most cases not well defined. There does not appear to be a distinct membrane even when the two masses of cytoplasm are seen to be clearly separable. These male cells are not organized in definite cells as they are in cycads and *Ginkgo*.

At the time of division the two nuclei are of approximately equal size. They may both develop in similar fashion and share the cytoplasm equally (figs. 3 and 4), or one of them may degenerate even after the partial division of the cytoplasm. Fig. 6 shows a male cell or sperm clearly bounded by a limiting membrane and with the nucleus at one extremity. Across it may be seen the faint line of demarcation between the two original cells. One nucleus has degenerated and only the faintest trace of it can be distinguished in the tail opposite the functional nucleus. This degeneration is probably fairly common, for one often finds unusually large male cells in tubes in which no trace of the other one can be found. It is possible, of course, that it has retreated up the tube and into the extra-nucellar portion and so escaped observation. I incline to the opinion, however, that it has either degenerated or slipped out of the cytoplasm and become so reduced in size as to be indistinguishable from the prothallial and other nuclei.

In a previous paper (3a) it was stated that these male cells might possibly be motile. Since then many tubes have been dissected out in sugar solution in the attempt to prove this. The results have been entirely negative. They are apparently amoeboid (as for that matter are the male cells of some angiosperms), but there are no structural evidences of locomotor organs of any sort, nor were any rapid movements of any kind observed. Fig. 5 shows a binucleate body cell rounding a sharp corner of the pollen tube at the point where it turns back into the nucellus after having crept along over the surface of the scale. The distortion in shape is not in any way due to crowding, for there was abundant room for

it to have passed the corner without having altered its shape in the least. It was evidently creeping along the convex side of the tube and was killed just as it was rounding the corner. That the male cells actively change position and shape on their own initiative is shown also by the manner of entering the archegonium, to which attention will be called in a succeeding paragraph.

In the account of the archegonium already published (3b), it was stated that no ventral canal cells or nuclei had so far been observed, but that it was unlikely that they were not formed. Archegonia have since been observed in which such a ventral canal nucleus (figs. 7 and 8) had been cut off. There still remains some doubt whether this nucleus is regularly cut off. All the cases observed were in gametophytes some of whose archegonia had already been fertilized. I have not yet found a gametophyte with archegonia unfertilized where ventral canal nuclei were present. Two explanations are tenable. Either this division is delayed almost up to the time of fertilization, and the canal nucleus degenerates very quickly so as to leave no trace of itself in fertilized archegonia, or it does not occur normally, but only in those archegonia in which fertilization has been delayed beyond the usual time. Though the latter appears the less probable supposition on general grounds, the evidence available is more in accord with it. In one case (fig. 8) two small nuclei were present. The egg nucleus in this case appears small. What would ordinarily be taken for the ventral canal nucleus is larger than in fig. 7. In the majority of cases the cytoplasm of the egg in which ventral canal nuclei were found appears to be undergoing degeneration. It clumps together and has very indefinite structure. It cuts with difficulty. In some archegonia, of normal appearance in other respects, there was present a zone of fibrillar cytoplasm surrounding the very large nucleus. These archegonia were also invariably found in gametophytes with one or more that had already been fertilized. EAMES (8) reports the regular formation of the ventral canal nucleus in *Agathis* immediately before fertilization, and its rapid degeneration. COULTER and LAND (6) were unable to demonstrate its formation in *Torreya*. The expectation, therefore, is strongly in favor of its being formed normally before fertilization.

I have not been able to decide from the evidence at hand which is the more probable hypothesis.

In preparation for fertilization the egg nucleus enlarges considerably. Fibrillar cytoplasm is not uncommon in mature eggs, and sometimes, as pointed out in a preceding paragraph, forms an inclosing sheath when for any reason fertilization is delayed. The fibers of these sheaths run tangentially to the nucleus and remind one somewhat of the development of spindle fibers. They are much more abundant than is usual in the development of a multipolar spindle, and furthermore there is no reason to suppose that these mature egg nuclei are about to divide, unless for the formation of the ventral canal cell nucleus. I have seen no evidence whatever of their actually developing a spindle. Moreover, the archegonia appear to have passed their maturity already. Yet, in view of the uncertainty of the ventral canal nucleus being cut off, this possibility cannot be entirely excluded.

The nuclear membrane is well developed and incloses a relatively small mass of chromatin distributed on a fine linin network. The whole is immersed in a large volume of nuclear sap. Fig. 9 shows these facts very clearly, except that the wrinkled condition of the nuclear membrane doubtless indicates that considerable shrinkage in volume has occurred through the application of reagents. The chromatin is distributed in more or less definite strands of beadlike masses on the very delicate linin. The total number of these chromatin masses in a nucleus is very large. The total mass is also surprisingly large when one considers its volume in the fusion nucleus and in the first two nuclei of the proembryo.

Fertilization

The archegonia are mature and ready for fertilization in California about the last week of March or the first week of April. At this time the egg nucleus is usually situated a little above the middle of the archegonium. The egg cytoplasm has almost completely filled the archegonium. The vacuoles that were so conspicuous in the earlier stages of development have all disappeared. Neither starch nor other form of stored food seems to be present in the cytoplasm. As will be shown presently, the cytoplasm

itself is used up in the growth of the proembryo. The neck is composed of about 12 cells arranged in a single tier (usually). The nuclei lie at the larger peripheral end of the cells. In the pointed central end there is little cytoplasm. Often there appears to be a slight opening among the neck cells, as if in anticipation of the entrance of the sperm.

The course of the pollen tube after reaching the female gametophyte is not always direct. It frequently wanders along between the megaspore membrane and the gametophyte, eroding the latter more or less, before turning down into an archegonium. Archegonia that are apparently mature and ready for fertilization may be passed and the male cells delivered to archegonia some distance farther away from the point of entry of the pollen tube. Very commonly the archegonial chamber above each archegonium becomes overgrown, so that the tube must force its way down to the neck. Some cells are destroyed in its approach, but the way is more often prepared by the thrusting aside of the intruding cells and the consequent opening up of the previously existing passage.

When the tube reaches the archegonium its tip is thrust down into the immediate neighborhood of the neck cells. The tip is then ruptured and the male cells crowd through the narrow passage of the neck. Sometimes both male cells enter and more frequently only one can be found. The archegonia are often so crowded with cytoplasm that the entrance of the male cell causes the extrusion of some of it through the neck (fig. 10). The entry appears to be violent, for the egg nucleus is commonly driven to the bottom of the archegonium and may even be driven through the bottom. The violent displacement of the egg nucleus shows very clearly that the male cells move with considerable force, while the extrusion of the cytoplasm seems to prove that they are not forced in by the tube, as has been said to be the case in certain other gymnosperms. It seems clear from the facts just stated that these male cells are actively motile, although it is almost certain that they have no cilia or other organs of locomotion. Their large size as well as their vigor of movement makes them conspicuous among Coniferales. Such size and movement are matched only among the cycads and *Ginkgo*.

After the contact of egg nucleus and male cell the cytoplasm of the latter gradually envelops them both. Fig. 11 shows a case where both are completely enveloped in a common cytoplasm even before the nuclei have begun to fuse. In figs. 12 and 13 each nucleus is accompanied by a distinct cytoplasmic sheath even though fusion is far advanced. The egg cytoplasm is generally much disturbed by the passage of the male cell through it and does not ordinarily recover its structure. The cytoplasmic sheath around the fusion nucleus, on the contrary, continues to grow rapidly, apparently at the expense of the general cytoplasm of the egg. With the growth of the proembryo the egg cytoplasm gradually disappears, until there is commonly very little of it when the walls are formed in the former. In some cases (upper right of fig. 32) the mass of dense cytoplasm surrounding the proembryonic nuclei becomes delimited from the egg cytoplasm by a distinct membrane. So far as I have observed, this membrane has nothing whatever to do with the walls of the upper tier of the proembryo, which form later and entirely within the limits of this membrane. Fig. 31 shows a small portion of this membrane in the upper part of the figure, just above the largest cell shown. The left-hand cell shows distinctly the beginning of the formation of the walls. The wall is less clearly shown in the other cells, though the plasmatic membrane around their dense cytoplasm shows clearly where it will form. This membrane does not always form, and I am unable to see any significance that may be attached to it. The cap of cytoplasm spoken of by EAMES (8) as occurring above the upper tier of nuclei in *Agathis* is not ordinarily present in *Araucaria*, though fig. 27 shows a band that might be interpreted as such a structure. This figure also shows very clearly the previous delimiting membrane over the upper surface, in fact extending over that part of the embryo formed by the previously mentioned cytoplasmic cap. No distinct membrane is to be seen in figs. 25, 26, and 30, even though there is sharp distinction between the egg cytoplasm and that of the proembryo. A membrane entirely around the proembryo is shown in figs. 28 and 29. The younger stages apparently do not possess membranes of any sort.

When the male cell enters the archegonium it consists of dense cytoplasm inclosing a solid and compact nucleus. It rapidly enlarges before actual contact with the egg nucleus. At the time of contact there is much more nuclear sap and apparently rather less chromatin. The condition of the nucleus before contact is fairly well shown in fig. 16, showing (above the 2-nucleate pro-embryo) the second male cell. This male cell is beginning to degenerate and is in consequence somewhat more dense and homogeneous than the functional one. It should be compared with figs. 11–15. The egg nucleus contracts instead of expanding. It also appears to lose much of its chromatin. A comparison of figs. 11–15 with fig. 7, all photographed at the same magnification, will make this point clear. At the time of fusion the two nuclei are not very different in size. It is rather difficult to obtain an accurate notion of their comparative sizes because of the markedly different shapes. The egg nucleus usually remains round (figs. 11–14), while the sperm nucleus becomes concave on the side pressed against the egg nucleus. In consequence, it spreads out laterally so as to cover a third to a half of the surface of the egg nucleus. Its change of shape is accompanied by a loss of nuclear sap, but probably not of chromatin. The two nuclei remain in contact for some time, as shown by the frequency of this stage in my preparations as compared with some other stages. The manner of fusion is shown in fig. 15. A perforation between the two nuclei is formed and the gap stretches until the contents of the two nuclei are contained in a common cavity. The nuclear membrane of the fusion nucleus thus consists of parts of both the sperm and egg nuclear membranes.

The chromatin of the two nuclei enters the fusion nucleus in the form of coarse or fine nets. Fig. 15 shows the chromatin as fine granules distributed evenly throughout the two nuclei. Fig. 13 shows granules arranged in series that might easily correspond to individual chromosomes. Other preparations show various gradations between these two extremes. Whether the two masses remain separate, as is said to be true in *Pinus* (5, 9a, 14, 15) and some other gymnosperms, could not be determined from the available material. No preparation showing the fusion nucleus after

complete fusion and before complete division was secured. I have been forced to the conclusion that this stage must be of very brief duration.

The relative position of the two sexual nuclei varies somewhat in different archegonia. The male cell probably comes in contact with the upper side of the egg nucleus. In many cases this relative position is shifted through the violence of the impact, so that the male cell may lie more or less to one side or even far around toward the bottom (fig. 13).

The second male cell sometimes enters along with the functional one. I have seen no indications of its functioning in the manner reported for *Agathis* (**8**), or in any other manner. When it enters it soon degenerates (fig. 16). I have seen no evidence that it ever divides, as has been reported for some other conifers (**9a, 9b**).

Attention has been called to certain peculiar bodies in the cytoplasm around the fusing sexual nuclei and sometimes in that of the 2-nucleate proembryo. Fig. 15 shows two of these bodies. The one to the left may possibly be a disintegrating vegetative nucleus from the pollen tube, though I do not think so. The one lying in the cytoplasm between the nuclei certainly is not of this nature. They are not found in every cell, but occur frequently enough to be legitimate objects of curiosity. They suggest the blepharoplasts of the cycads. When they were first observed a diligent search was instituted immediately for similar structures in the body cell and the male cells before they enter the archegonium. The results were entirely negative. Fig. 17 shows one in the second male cell within the archegonium. The division of the fusion nucleus has not been observed, and it is possible that they may function here as blepharoplast-like or centrosome-like bodies.

Proembryo

The division of the fusion nucleus probably follows soon after the complete union of the egg and sperm nuclei. The resulting nuclei may lie one above the other, side by side, or in an oblique plane (figs. 17–19). They vary considerably in size, as is evident from a comparison of figs. 18 and 19. Before the next division there is a moderate increase of cytoplasm. The two nuclei

probably divide simultaneously, since no 3-celled proembryos were found. No mitoses in the proembryo have been observed, nor any trace of evidence that the nuclei divide amitotically. I have already, in former papers (**3a, 3b**), called attention to the very curious fact that almost no mitoses in the critical stages of development of *Araucaria* have been observed. It is a very curious and puzzling fact, not to say a very annoying one. SAXTON has recently called attention to a similar state of affairs in another southern hemisphere form, *Actinostrobus pyramidalis* (**18a**).

The four free nuclei may occupy almost any position with reference to one another. It has already been mentioned that the position of the fusion nucleus appears to depend on how much it is displaced through the violence of the contact between egg and sperm. It may lie near the middle of the archegonium, as it does in *Agathis* (**8**), or more generally near the bottom. The succeeding divisions take place wherever the fusion nucleus has been left. This same displacement would probably tend to conceal any polarity that the fertilized egg might possess. The commonest appearances of the proembryos are shown in figs. 20 and 21. Sometimes the four nuclei may all lie at the bottom of the proembryo, as in figs. 23 and 25. The subsequent divisions do not appear to follow any definite order nor are they simultaneous. Whether the 4 nuclei were tetrahedrally placed (figs. 20, 21), placed in a single vertical plane (fig. 23), or in a curved line around the bottom (fig. 23), or in any other position, seems not to affect the ultimate result. Irregular division continues for two weeks or more before the final arrangement of the cells in tiers. The number of cells or free nuclei at this time varies considerably. No counts of less than 32 nor more than 45 were obtained from an examination of a considerable number of embryos of about this stage. The number of proembryos showing the beginning of wall formation was so small that it cannot be certainly said that some of those with 32 free nuclei might not have had more at the time of wall formation. Many of these seemed as large and as definitely arranged as the ones with a greater number of nuclei. It seems to me, therefore, that the number of nuclei at the time of wall formation is probably variable.

After or just about the time of the cessation of free nuclear division the nuclei arrange themselves as shown in fig. 30, which is a median vertical section. It will be seen that there is a central group of nuclei arranged more or less regularly in two tiers, surrounded by a complete jacket of peripheral nuclei. These peripheral nuclei are usually more numerous on the lower side than on the upper. Even before walls are formed the lower nuclei sometimes begin to elongate, foreshadowing the formation of the cap. Fig. 32 shows a proembryo in which walls are forming about the lower nuclei, which are already set off in definite cells. The walls appear to form first in that part of the embryo which first begins elongating. In fig. 32 the lower cells formed first and began elongating while there is yet no indication of the future cells in the upper portion of the proembryo. Precisely the opposite state of affairs is shown in fig. 31, where the upper cells are elongating and forming walls while the lower cells are just forming but have not begun elongation and have only faint traces of walls around some of them.

After the complete establishment of walled cells the elongation which had already begun continues simultaneously in both the upper and lower cells. It is only after this elongation that one may properly speak of tiers, for, as already pointed out, the cells are arranged concentrically rather than in layers. Few or no divisions occur in the terminal group of cells, destined to form the cap, during this preliminary elongation, and none at all subsequently. There is a considerable increase in the upper group. They divide longitudinally, so that there are ordinarily about twice as many cells in the young suspensors as there were in the group of cells from which they were developed. The number shown in cross-section varies somewhat, but is usually not far from 20. As the suspensor cells elongate, their upper ends are thrust backward and upward (that is, in the direction of least resistance) until they encounter the firm top of the archegonium. Their upper ends ordinarily become swollen during elongation, so as to stretch the upper part of the archegonium (fig. 33). Incidentally this figure also shows that the neck is not ruptured by the entrance of the male cells and is not torn away from its mooring to the

upper part of the jacket as EAMES has shown to be the case in *Agathis* (8).

The cap is completely organized by the time the elongating suspensors have reached the neck of the archegonium. Owing to the greater elongation of the central cells of the cap than of those in each successive circle back of it, the cap has a much more pointed appearance than the proembryo at first exhibited. Fig. 35 shows a mature cap. It exhibits very clearly the relations of the component cells. This figure also brings out very clearly the fact that the tiered appearance of the embryo is more apparent than real, for the cap is really formed of all the cells of the peripheral layer below the suspensor. The embryonic group lies in a cup-shaped depression in the top.

The embryonic group of cells consists of a hemispherical or globular mass of small cells. There are usually 20–24 cells in the hemispheres (fig. 35), but there may be as many as 30 or even more in the globular masses. The number contained in the proembryo remains unchanged from the time they are set off and walled in until after the development and elongation of the primary suspensors.

After the organization of the walled proembryo and its preliminary development of the cap and an anchorage in the top of the archegonium, the suspensor cells begin a rapid elongation, accompanied by transverse division. This pressure of elongation maintains a firm contact of the cap with the cells in front of it. The suspensors at first thrust straight downward toward the center of the endosperm. This stage of development is probably accomplished quite rapidly, for most preparations show either free-nuclear proembryos or long, coiled suspensors (fig. 37). Usually more than one embryo starts development, about three of which start near enough at the same time to make the race for position in the center of the endosperm (fig. 36) a spirited one. When they have reached the center, the competitors coil around one another in the struggle for supremacy. One finally emerges below (fig. 37) —the victor. The others ordinarily perish without further development, though not a few cases have been seen where a second embryo had reached some such degree of development as that shown in

fig. 40 or 41. I have found no seed with a second embryo large enough to be seen with the naked eye.

Ever since STRASBURGER'S account (**23**) of the cap of the proembryo of *Araucaria brasiliensis*, it has excited comment on its apparent specialization. It has been spoken of as a protecting cap (**7, 20, 23**), but no evidence has been adduced to show that protection is at all necessary. The caps are not made of specially strong cells, nor do they show any effects of abrasion, which might reasonably be expected if they were of use as a protection. Neither do the cells of the endosperm surrounding the caps appear to have been crushed and thrust out of the way. It seems much more likely that the cells of the cap secrete a digestive enzyme. An inspection of figs. 34–37 will make this evident. Very few cells of the endosperm show any distortion from crushing, while practically all of them show the action of some corrosive agent on their contents or even in some cases on the walls themselves. I have seen no evidence, however, for thinking that the secretion of enzymes is limited exclusively to the cap. In fig. 34 it will be seen that the region of greatest cell destruction is around the embryonic region and not directly in front of the advancing tip. It is clear from fig. 37 that the cavity in which the proembryos lie continues to enlarge around the suspensors long after the cap has passed by. To put the argument in another way, the cavity should be cylindrical if solution occurs only around the cap, whereas the cavity is actually shaped like a wide-mouthed cone, showing that solution has gone on all over its surface and not merely at the apex of the cone. It is, of course, possible that this might be true and still all of the enzymes be formed in the cap, but excreted in such abundance that they fill the entire cavity with a solution of equal strength. I suspect that the matter comes to about this. The cap looks like a highly specialized structure and should in consequence have a specialized function. The proof that it does actually have a special function has not yet been adduced.

Sometime in June or July the primary suspensors have reached their limit of elongation. Then begins the third and final stage of development of the proembryo. The activities of this stage are limited to the embryonic group of cells. A rapid multiplication

of its cells is the first step. Fig. 38 shows an early stage in this growth. As soon as it begins the cap cells begin to disintegrate and are soon crushed (figs. 38 and 39). At first all the cells divide with equal rapidity. Very soon the upper cells show a tendency to enlarge, and more especially to elongate, while the lower ones continue division unabated. An early stage of this phase of development is shown in fig. 39 and a later one in fig. 40. The proembryo now consists of two regions: (1) The very actively dividing cells at the tip constitute a large apical meristem, and (2) the cells behind the meristem gradually cease division and elongate so as to produce a massive secondary suspensor which pushes the proembryo still farther down into the endosperm. After a time (a month or so) the proembryo consists of a massive suspensor and a large cylindrical body of meristematic tissue. The activity of the apical meristem practically ceases.

Embryo

Three new meristems are now developed. The first of these is picked out where the suspensor joins the main body and is to form the growing point of the hypocotyl. The other two form either side of the original growing point and quickly develop the two cotyledons. The remains of the primary meristem constitute the meristem of the stem apex, which continues dormant until some time after germination of the seed. Fig. 42 shows a longitudinal section of such an embryo some time in early September. All the regions of the embryo are now in course of development. Stem and root apices, cotyledons, and vascular tissues are clearly in evidence. These regions continue growth for two months or more before the seeds have reached the shedding stage. Growth in this period is largely confined to the cotyledons, which become very large in comparison with the hypocotyl.

The distribution of the vascular tissues in the embryo is shown in figs. 42–44. The cotyledons are traversed by 7 vascular bundles. Each of these can be traced backward to its separate union with the vascular cylinder of the hypocotyl. In the latter the procambium strands form a hollow cylinder. Just below the origin of the

bundles of the cotyledons the vascular cylinder is more or less quadrangular. The longer axis lies in the plane of the cotyledons. The other two sides are more weakly developed and bend in slightly toward the stem apex. The vascular cylinder rounds up gradually as it extends toward the root.

Resin canals are abundant in the cortex of the embryo, but do not occur in the pith or wood. There is a fairly regular circle of them three or four cells beneath the epidermis. Another definite circle occurs just outside the procambium strands. There is no definite boundary between stele and cortex, and so I am somewhat uncertain whether this ring of ducts should be attributed to the cortex or to the pericycle. Some authors (20) apparently speak of all the outer portion of the embryo as pericycle. I can see no good reason for this usage. The tissue is all alike at first. Then the procambium strands arise in the central region, inclosing a region of parenchymatous cells and are in their turn surrounded by a similar parenchymatous region. There is a pretty regular correspondence in number and position between the procambium strands and the resin ducts. Between the inner and outer circles there are numerous other less regularly disposed ducts. The preceding facts are shown in fig. 43, though not so clearly as I should have liked. Resin ducts occur in the cotyledons also. In the base they accompany the vascular bundles and are just below the epidermis of the outer face of the cotyledon, but not on the inner side of the bundles. Farther out toward the tips the outer ring extends completely around beneath the epidermis, in much the same way as in the hypocotyl. Resin ducts do not appear to extend upward in the embryonic mass from which the final embryo is differentiated beyond the dark line shown in the upper part of fig. 42.

In the subsequent growth of the embryo the hypocotyl changes very little, while the cotyledons elongate enormously. At the time the seeds drop from the cone axis (late November to January), the embryo is about 3 cm. long, of which the hypocotyl forms about 5–6 mm. At this time the embryo is quite straight and extends to within about 1 cm. of the tip of the endosperm. The hypocotyl is crowded closely into the apex of the seed.

After the fall of the seed the embryo continues to grow unless it becomes excessively dry. Fig. 48 shows a longitudinal section of a seed that had been stored in a tin box in my laboratory for a year and a half at least, and possibly two and a half years. The seed has continued the development of the embryo in much the same manner that would have occurred if it had been planted, except that development has gone on at a much reduced rate. Many other seeds in the same box put out roots 3–4 inches long within 6–8 months. When planted the hypocotyl emerges in the spring following the winter in which the seeds were shed. As these seeds are often shed in California before the rainy season has begun, it is evident that this intraseminal development is a means of enabling them to make the most of the growing season when it does come. It is not unlikely that in their native habitat this habit is equally useful. The seeds that did not continue growth appeared not to have done so on account of the attacks of a fungus that reduces the endosperm to a fine white dry powder. The embryos become yellow, shrunken, and waxy. The proportion of seeds failing to sprout was much the same in the box on my shelf as when they were properly planted. In fact, complete burial seems to be unfavorable to successful germination.

Endosperm and seed

During the development of the embryo important changes occur in the female gametophyte. The young gametophyte consists of comparatively large, very thin-walled cells with exceedingly scanty contents. They are multinucleate at the time of fertilization. By the time the proembryo has used up the food supplies of the archegonia and has begun to push down into the gametophyte, the cells immediately below have increased their cytoplasm markedly (figs. 34, 36, 37). The nuclei also increase in number. As the embryo advances the zone of food formation and storage precedes it. In the center of the endosperm there is left a narrow space more or less free from food storage. Toward this latter the embryos direct their course. The region of growth and storage of foods is below the archegonia. The upper region shrivels up and is crowded back into the apex of the developing seed. The

lower part of the gametophyte enlarges many fold. This growth is due in part to cell multiplication, but more largely to the increase in size of the already existing cells. As they enlarge they form and store up starch and multiply their nuclei up to 4 or 5 in almost every cell, and in some of them to twice these numbers. Fig. 46 shows nearly all of a single cell taken from about the middle of the endosperm. The light lines near the border mark the position of the delicate cell walls, which do not show in the photograph. The large oval bodies are starch grains, and the small round ones are proteids. Many of the cells are so crowded with food as to make photographs difficult. The proteid granules appear much later than the starch grains. They are not distinguishable optically much before the stage of the embryo shown in fig. 42. They never become so large or so numerous as the starch grains. Much the larger part of the growth of the gametophyte occurs during this period of food formation and storage subsequent to fertilization. At this period it is not more than 5–6 mm. in length, while at maturity it is about 4 cm. long and 15 mm. wide at the widest part. At fertilization it is broadest just below the archegonia; at maturity it is broadest at the basal end (compare fig. 48 with fig. 4 in the earlier paper [3**b**]).

After the embryo has differentiated its organs and has begun its final stage of development its cells become packed with food materials (fig. 45). The smaller round grains shown in the figure are starch. The proteids occur in very large subspherical masses. Not infrequently the large globule includes a smaller one. The inclusions are also sometimes angular and probably crystalloids. The latter are smaller than the globular ones. Peculiar dumb-bell-shaped bodies are also found included in the large proteid masses. Often one end is included, while the other projects freely from the surface. The nuclei of these cells often become very large and sometimes flattened. Two conspicuous nuclei of this sort are shown along the lower side of fig. 45.

The growth of the gametophyte does not destroy the nucellus, as usually happens among the gymnosperms. On the contrary, it continues to develop *pari passu* and forms an integral part of the mature seed coat. In fig. 48 it can easily be distinguished as a

separate layer of the seed coat, especially on the right side of the figure. Its tissues become lignified in precisely the same manner as those of the integument and scale. Fig. 47 shows a section through the developing testa at about the time it begins to become woody enough to be unsuitable for cutting in paraffin. The outer layer consists of a conspicuous epidermis filled with mucilaginous contents. Beneath this there is an irregular layer of cells with darkly staining contents, probably largely tannins. On the inner border next the nucellus there is a less conspicuous epidermis underlaid by several layers of elongated, thin-walled cells with very scanty contents. The larger part of the testa consists of the irregular cells shown in the central part of the figure. These cells become elongated and more tangled as the seed grows larger. At first their walls are very woody and tough, but not at all brittle. In the adult seed they turn brown, become much more brittle, and when dry are capable of being in part reduced to fine brown powder by crushing. The changes in the integument and nucellus are of the same kind as those occurring in the scale itself. The result is that in the mature seed all these parts have developed into a homogeneous structure, and ovule and scale have united to produce the seed. It resembles what might be expected to develop from a naked anatropous ovule.

Discussion

Araucaria and *Agathis* resemble one another very closely, differing only in minor points. They present a number of sharp contrasts to most other conifers. Pollination of the ovuliferous scale, very long and extensively branching pollen tubes, extruding nucelli, precocious division of the body cell, large actively motile male cells, and concentric proembryos will serve to recall some of these points of difference. Excepting *Saxegothaea*, with its protruding nucellus near which the pollen germinates, these features are very different indeed from the corresponding ones in the other families of Coniferales.

These resemblances to *Saxegothaea* have attracted the attention of a number of botanists (**16, 22, 24, 25a, 26**). Taken in connection with other resemblances they are sufficient to create a strong

probability of a real relationship between the araucarians and podocarps.

Though it has been generally recognized by botanists that the protruding nucellus is correlated with the method of pollination and extensive growth of the pollen tube, it does not appear to me that this very peculiar situation has received anything like the attention that it deserves. I have elsewhere (**3a**, **3b**) expressed the opinion that pollination of the scale, coupled with an extruded nucellus, is more likely to indicate the retention of an ancient habit than the acquisition of a new one.

It must be admitted that we know comparatively little about the structures and affinities of paleozoic seeds and pollination devices. In the absence of present knowledge we must resort to more or less probable conjectures in our attempt to relate the already known facts. We do know enough, however, to make it very probable that the earliest known gymnospermous seeds are very far from being representative of the beginnings of the seed habit. They had already acquired numerous complexities. It is scarcely credible that the actual first seeds should have been provided with a deep and narrow micropyle, with devices to draw the pollen grains down into it and on into a chamber specially prepared for their reception by the breaking down of the cells of the nucellus. It is further to be noted that seeds of this type have in their pollen chambers pollen grains that show no signs of having possessed pollen tubes. It seems evident that this complexity of devices must have had a more or less extended history, and that to understand it we must try to conjecture the conditions and structures that would have been likely to be developed as intermediate stages between heterosporous pteridophytes and these paleozoic gymnosperms.

It is not alone that we do not know the history of the seed structures of these early gymnosperms that makes the problem difficult. The difficulties of relating the structures known in more modern plants to these ancient ones is no less difficult.

An analysis of the known facts will show that there are four distinct methods of accomplishing pollination and fertilization now known among gymnosperms: (1) the Cordaitales and Cycadofilicales

have a pollen chamber in the nucellus in which the pollen grains lodged; no pollen tubes are known and the indications are that they were not developed; (2) in Cycadales the pollen lodges in an already prepared pollen chamber in the nucellus and forms haustorial branching pollen tubes, which do not penetrate toward the female gametophyte and take no part in transferring the ciliated sperms to the archegonia; they are haustorial and nutritive in function; the way for the sperms is cleared by the gradual dissolution of the cells forming the bottom of the pollen chamber; (3) in most of the Coniferales the pollen passes down to the tip of the nucellus, where it puts out a pollen tube that is both nutritive and a sperm carrier; (4) the Araucarineae, and to a less extent *Saxegothaea*, are pollinated on the ovuliferous scale at a distance from the ovule, from which point a pollen tube grows toward the micropylar end of the ovule and there enters the protruding nucellus.

Another fact that seems to me especially significant in any attempt to account for the origin of these various habits is that in *Araucaria*, some podocarps (13) related to them probably, and in cycads, the embryo is not mature when the seeds are shed and keeps on growing after the seeds fall. It appears to me that this is the sort of habit one would theoretically expect to find in primitive seeds for reasons stated below. It adds strength to this supposition that Cycadales are universally recognized to be primitive plants, and that many investigators believe the araucarians to be the modern representatives, little changed in many ways, of a very ancient line and to be closely connected with the podocarps. Perhaps it will be worth while to attempt a brief analysis of the possible origins of the four classes of pollination devices mentioned above.

CYCADOFILICALES.—Whether the Cycadofilicales are more primitive than the Cordaitales is a debatable question, but that they exhibit their seeds on less modified foliar organs affords some reason for thinking that the seeds themselves are also less modified. *Physostoma elegans* (17) will serve as a starting-point in an attempt to work back to the origin of seeds of this type. In the seeds of this type the integument is split up into more or less divergent lobes

which do not closely invest the nucellus. The female gametophyte is covered by a very thin layer of nucellar tissue above. The pollen chamber occupies almost all of the exposed portion of the nucellus and probably laid bare the gametophyte at its maturity so that the free-swimming (probably) sperms had direct access to the archegonia.

One may suppose that when pollination first began the nucellus was freely exposed, and that the integument was either wanting or less developed than in *Physostoma.* Since these seeds were freely exposed on leaflike organs, there must have been developed, as the first necessary step to pollination, a sticky secretion on the nucellus to catch the microspores or pollen grains. It must be further supposed that the pollen grain was able to secure sufficient food from this secretion to maintain itself for such a length of time as was necessary for its further development, and until the gametophyte had broken through the nucellus and exposed the matured archegonia. It is supposable that the processes that produced the sticky secretion might in the course of time develop the habit of further destroying the cells of the tip of the nucellus to produce a rudimentary pollen chamber. The further step of eroding this chamber deep enough to allow access to the archegonia without waiting for the growing female gametophyte to rupture the nucellus would appear to be easy and logical.

During the development of the pollen chamber the integuments would be developing in the direction of greater efficiency in securing the deposition of the pollen on the tip of the nucellus. As they closed up the sticky secretion would be exuded as a pollination drop to catch the pollen. If the ovules stood upright gravity would effect the delivery of the pollen to the pollen chamber. In any case, the pollen would probably be retracted along with the pollen drop when it began to dry up. It is in this stage of development that the seeds of Cycadofilicales are found fossil. The reason (it appears to me) is that seeds that had been fertilized (or were far enough along to be fertilized soon) fell to the ground and continued their growth. If this were the case one would expect to find fossil only those seeds that had fallen too soon to be able to continue growth.

CYCADALES.—It appears that the cycads, ancient and modern, are closely related to Cycadofilicales. This relationship is not contradicted by the pollination devices, for they are so similar as to afford little difficulty in bridging over the gap. The method of deposition, as well as the presence of a pollen chamber in the nucellus, are as near alike as one could well expect. The greatest difference is the presence of a pollen tube in modern cycads. It has already been pointed out that this tube grows away from the female gametophyte and is exclusively haustorial in function. The pollen chamber itself provides access to the archegonia in just the way that we have conjectured for the preceding group. The real difficulty arises in supplying a convincing reason for the origin of a tube at all. If we are to homologize the pollen tube of cycads with the rhizoid of the germinating spore of their pteridophyte ancestors, it means that an organ that had been completely lost has been revived. Admitting the possibility, about which I am very dubious, of the revival of this ancient structure after the lapse of geologic ages, it is evident that it would be useful, subject to the laws of selection, and likely to be preserved. If pollination preceded fertilization only a short time in the earlier seeds, and the remaining processes took place on the ground, it is evident that what these ancient plants had attained was not the "seed habit," in the sense that we employ the term with reference to modern plants. They had merely attained the ovule and pollination habit. A real seed could be developed only if the seed structure were retained on the plant until its maturity (except the growth of the embryo itself). An advantage would certainly lie in early pollination of the ovule that would permit the further growth of the ovule even while the gametophytes were maturing. That this habit of pollination long before fertilization is an advantage is indicated by the fact that all modern seeds practice it, although it is difficult to imagine that the first seeds or ovules that were pollinated furnished food and protection on the exposed nucellus sufficient to maintain the male gametophyte for a year or more, as is commonly the case in modern conifers.

CORDAITALES.—The Cordaitales differ from the Cycadofilicales, among other things, in that their seeds occur in cones and not on

exposed foliar organs. It is an interesting and I think a significant question whether the pollination habit or the cones were developed first in this group. If we assume provisionally that the cone habit did not develop until after pollination was a fixed habit, the explanation of the origin of the latter given above might be applied to this group also. The cone habit would then have been acquired while the integuments and pollination apparatus were being perfected. The difficulties of this explanation seem to me not to lie in its application to the Cordaitales themselves, but in the assumptions that must be made in deriving the Coniferales, particularly the Araucarineae, from them.

If it be supposed that the Cordaitales as a class had all reached essentially the same stage of development of the pollination devices, and that it was comparable to that already described for the Cycadofilicales, we may then seek to see just what changes must have taken place during the evolution of modern conifers. *Ginkgo* presents almost the same devices as the cycads, and we may therefore confidently assume that an explanation that will suffice for the one will prove adequate for the other.

CONIFERALES.—Excepting for the moment the araucarians and *Saxegothaea*, the modern conifers are characterized by the pollen being caught in a pollination drop and drawn down upon the tip of the nucellus or at least into the micropyle, where it germinates. The pollen tube that is produced is both haustorial and spermiferous. It grows more or less directly down through the nucellus and delivers the male cells in the neighborhood of the archegonia. It must be noted that it thus differs very sharply from the pollen tube of the cycads and *Ginkgo*, where the pollen tube is strictly haustorial and is never even entered by the body cell or its products. It is evident therefore that *either this pollen tube is one that has altered its function and completely changed its method and direction of growth or it is a different kind of pollen tube.*

As there were no pollen tubes (probably) in the Cordaitales there is no compulsion to assume that their descendants necessarily developed a tube that behaved in the manner of the cycads. *Ginkgo*, of course, would be an exception to this statement, but

may be left out of present consideration because there is little evidence that it lies in the line of direct descent to the conifers. Since the method employed in the cycads is an entirely successful one (more so than that of the conifers, in fact), there would appear to be no reason why variations from it in a direction that would be not only of no use to the plant but a positive hindrance to it would be selected and preserved, even if they should occur. It seems very unlikely, therefore, that such a change of function did occur.

If we start with the condition actually found among the Cordaitales, where pollen grains without any tubes were deposited in a pollen chamber in the nucellus, can we see any sufficient reason for the giving up of the pollen chamber and the development of the tube? One type of ovule is just as easy to pollinate as the other, for if pollen can be gotten to the nucellar tip, there would appear to be no difficulty in getting it into the pollen chamber. If it reached the pollen chamber safely and the pollen chamber broke through so as to give the swimming sperms direct access to the archegonia, it is difficult to see what would be gained by the giving up of the chamber and the formation of a pollen tube. That the conifer method is in fact inferior and would be selected against is strongly indicated by the fact that the proportional number of good seeds in their cones is decidedly less than that of cycad cones. The evidence would thus appear to be against such a derivation of the coniferous pollen tube.

If it is difficult to see any adequate reason for the evolution of the ordinary coniferous pollen tube from the conditions found in the Cordaitales, it is vastly more difficult to imagine any adequate reason for its further evolution into the araucarian tube. We must imagine not only that the pollen chamber has been given up but that the place of pollen deposition has gradually retreated out through the micropyle and back along the scale from bad to worse. JEFFREY and CHRYSLER (11) would have us believe, not only that it did actually do this, but that to compensate itself for the disadvantage it was compelled to form extensive lateral haustorial branches and to "proliferate" the two "primitive" prothallial cells. I have not yet seen any reason advanced why the nucellus, having given up the habit of forming a pollen chamber, should have

undertaken to follow up the pollen grains by protruding itself through the micropyle. A theory beset with such manifest difficulties can be accepted only if no more probable one can be found.

ARAUCARINEAE.—The araucarians have been thought by some authors to be derived from the lycopods (**20, 4a, 4b, 22a, 22b**), and by many others to be derived directly from the Cordaitales. We have seen that the pollen tube structures do not lend any support to the derivation of the araucarians from the cordaiteans *through the other families of conifers.* Whether the pollen tube structures could be derived directly is a question that we can best attack after considering the bearing of these structures on the theory of a lycopod origin.

Miadesmia (**19**) and *Lepidocarpon* (**19**), two seed-bearing lycopods, seem to me to present the most suggestive analogies of the manner in which such a seed as that of *Araucaria* may have been evolved. I do not mean to imply that these analogies are sufficient or adequate evidence for deriving the araucarians alone or conifers as a whole from the lycopods, but merely that the araucarian pollination apparatus could be easily derived from such seeds as these plants possessed, whether they belonged to lycopods, cordaiteans, or what not.

The seeds of *Lepidocarpon* were formed in cones and not exposed as in the Cycadofilicales. The same is true of *Miadesmia.* I am inclined to attribute to this fact considerable importance. Seeds that originated on a naked foliar structure would necessarily have to be pollinated on the ovule to have any chance of success at all under any ordinary conditions of plant growth. Otherwise, the ciliated sperms would have encountered almost insuperable difficulties in reaching the archegonia and would have been limited to wet weather. It seems from such considerations that Cycadofilicales and their allies have been from the first pollinated on the nucellus, but no such compulsion rests on plants which had acquired the cone habit first. The natural, easy, and probable place for the lodgment of the earliest pollen would be between the scales anywhere. There would be far less danger of the pollen blowing away before it could become effective because of its protected

situation and far greater probability of frequently finding sufficient moisture for the swimming sperms. In fact, neither of these lycopod seeds shows any signs whatever that pollen ever lodged on the nucellus. *Miadesmia* actually possesses integumentary outgrowths that appear to be designed to prevent pollen from entering the micropyle. Though these hairs would probably keep pollen from entering the micropyle, they would serve equally well to catch it and retain it near the ovule. Sperms freed here would be in a favorable position to reach the archegonia with a minimum amount of moisture, which might very well be exuded by the cone scales, just as it is today in *Araucaria*. The reasons for the *formation* of pollen tubes in this type of pollination are no greater than in the previous type, but once formed and endowed with the habit of growth toward the archegonia, they would add immensely to the probability of fertilization, and so would tend to be selected and preserved in the evolution of the seed. Such tubes would probably always have grown toward the micropyle of the ovule because of the greater opportunity of securing suitable food in that direction. They would probably branch for the same reason that fungus hyphae branch (whatever that reason may be). Probably the main branch did not at first regularly reach the nucellus, but only came to do so later, after the nucellus had acquired the habit of secreting some chemotropically active substance. Then if the pollen tubes in search of food ever came to penetrate the nucellus before it had been broken through by the female gametophyte, they would furnish a more direct and easy route for the swimming sperms to the archegonia than for them to be freed outside the ovule as in the earlier stage. An advantageous habit of this sort would be likely to be preserved. We thus attain the state of affairs illustrated by the araucarians.

It is perhaps worth while pointing out that, in the above argument, *the various changes are not supposed to have occurred because they would be advantageous, but having occurred fortuitously to have been preserved because they were advantageous.* In contrast, the theory outlined above (11) of the derivation of pollen tubes among the conifers and araucarians requires *the derivation of disadvantageous changes and their selection and preservation notwithstanding.*

Moreover, it requires the further derivation of other structures (proliferation of the prothallial cells) to compensate for the disadvantages.

It is comparatively easy to derive the pollination apparatus of the ordinary conifers from the araucarian type by reduction, for it can be shown that each step would be an advantage, and so likely to be retained whenever it chanced to occur. In the first place, any change that would bring the pollen grains nearer the micropylar end of the ovule would shorten the distance to be traveled and so be an advantage. *Agathis* shows such a change, and there are abundant reasons for thinking it less primitive in other respects than *Araucaria*. *Saxegothaea* shows a still further stage of reduction, and there are also good reasons of other sorts for believing that it too is related to the araucarians and derived from them. The podocarps and pines illustrate the final stage where the pollen tube forming grains reach the inclosed nucellar tip before germinating. Once the pollen was deposited directly on the nucellus, changes tending to cover the nucellus by the integument and to draw the pollen down the micropyle by means of a pollination drop would be further advantages in the way of further protection to the germination tubes from drying, as well as some advantage in closing up the micropylar orifice in the maturity of the seed.

As we have seen that it would be easy to derive the Pinaceae from the araucarians so far as the seed and pollination habits are concerned, and next to impossible to reverse the order, we may now inquire whether it is possible to derive the araucarians from the Cordaitales directly in respect to the same structures. There are abundant evidences that among paleozoic gymnosperms of both great groups the nucellus either protruded from the nucellus or projected far into it. So far as I have been able to find from the literature available to me they all show pollen chambers. I suspect that this preponderance of evidence in the published accounts is due in part at least to the general opinion that pollen chambers are primitive, and so this feature has been exploited. It is conceivable, at any rate, that some of the paleozoic gymnosperms did not have pollen chambers and were not pollinated on the nucellus, but on the

scale. If such evidence should be forthcoming, the line of argument that has been used in connection with the lycopod seeds could be equally applied to the Cordaitales. The cordaitean seeds were formed in cones, and I should strongly expect that some of them were pollinated on the scale instead of the nucellus.

The theory would run something like this. When the seed habit was developed, the plants were in the midst of acquiring the cone habit. Pollination would therefore differ in nearly related plants. The ones that first perfected the pollination habit would be likely from the first to be pollinated on the nucellus. The ones forming cones first probably acquired thereby the habit of pollinating the scale. Some of these may have deposited the pollen so near the nucellus that they soon passed through the intervening stages and so show no special differences from those that always had had the pollen on the nucellus. The history of the pollen chamber would be the same as that already outlined for the Cycadofilicales. Whether these ancient plants that gave rise to modern conifers were more like araucarians or other modern conifers in other respects cannot, of course, be decided on these grounds. It does seem to me that the mesozoic conifers very probably did resemble the araucarians in respect to the seed and pollination habit. This might be equally true whether they resembled the araucarians in their vegetative structures or were more like the Abietineae, as has been vigorously maintained in recent years by some anatomists (10).

The theory of the pollen tube above outlined is applicable to the structure of the male gametophyte itself. There is nothing to be explained away, as must be done if we attempt to derive the araucarian type from the pine type (11). The numerous prothallial cells are not then to be thought of as something to be explained away, but as what is left of the ancient prothallus. A figure (2, fig. 2) from Miss BENSON'S paper on *Lagenostoma* seems to me to be capable of another interpretation than the one given by the author. The figure shows a number of pollen grains in the pollen chamber. At the upper right of the figure is a group of one large and several small cells. The large cell is labeled "a sperm," and the smaller ones are said to be probably fungal

cells. I should like to suggest that they strongly resemble a group of prothallial cells about a body cell or sperm as they appear in *Araucaria*. So far as it goes, it seems to me, the evidence is that there were prothallial cells in the paleozoic pollen grains, notwithstanding that eminent botanists have interpreted the evidence to the contrary (7).

I have elsewhere (3a) called attention to the very large male cells of *Araucaria*, and EAMES has recently (8) shown that they are present in *Agathis* also. A further peculiarity in their formation is exhibited by *Araucaria brasiliensis*, in that the division of the body cells occurs a long time before fertilization and not about simultaneously (7) with the division of the central cell a few days before fertilization. This division usually occurs outside of the nucellus a month or even two months before the pollen tube has actually reached the archegonia. Not only are the male cells long-lived, but they appear to be more active and independent than those of most conifers. This appears to me to be a primitive and unspecialized behavior, and one that would be unlikely to be derived secondarily from the condition now obtaining in the pines.

The male cells of *Araucaria* pass through the neck of the archegonium without injuring it. COULTER and CHAMBERLAIN (7) assert that the pollen tube of the Pinaceae destroys the neck, though LAWSON (12c) has recorded that the pollen tube of *Sciadopitys* passes between the neck cells. Among the Taxaceae the neck cells are sometimes destroyed (*Torreya*, COULTER and LAND 6), and sometimes the male cells pass through without injuring them (*Phyllocladus*, Miss YOUNG 26), just as in *Araucaria*. In *Agathis* (8) the male cells enter the top of the archegonium to one side of the neck cells, which are thereby broken loose from their anchorage to the jacket. In *Podocarpus* the necks appear to be broken through by the neck, though SINNOTT'S (21) statement is not specific as to whether the neck cells are destroyed or not. In *Cephalotaxus* (12b) the neck cells are probably destroyed by the entrance of the tube between them. In *Cryptomeria*, which externally resembles some species of *Araucaria* very closely and has other suggestions of affinity as well, the male cells are said (12a) to pass between the neck cells, but to injure them in doing

so. *Araucaria* finds again its closest resemblances among the Taxaceae. The habit of entering the archegonium between the neck cells without injuring them is an old one, dating back to Archegoniatae generally. The habit in the araucarians of liberating the male cells outside of the archegonium and allowing them to enter it under their own power of movement is doubtless more primitive than that prevailing among the Pinaceae, where the tube actually delivers the male cells inside the archegonium in many cases.

The female gametophyte is very similar to that of most conifers. Attention has been called to the apparently peculiar method of wall formation in the transformation of the free-nuclear state to the walled prothallus. Since that was written (**3b**), SAXTON has published an account of the life history of *Tetraclinis articulata* (**18b**), in which he shows a photograph (fig. 6 of his paper) in which three of the nuclei occupy a position in what is elsewhere supposed to be the wall of the forming alveoli. Possibly this may indicate that walls are formed in this plant in the same way as in *Araucaria*.

The multinucleate condition of the prothallus at fertilization time is not peculiar to *Araucaria*, being now recorded in several other genera (*Agathis*, *Cryptomeria*, etc.). It is probably more widespread than the literature indicates at present.

The late stage at which the ventral canal nucleus is cut off, and the lack of any trace of a wall are certainly not evidences of primitive behavior. In fact, there are very few evidences that the female gametophyte has lagged in its development behind conifers in general. That is, it seems to me, as it should be. The male gametophyte has retained a lot of primitive characters, because they are associated with the habit of pollinating the scale. These influences do not affect the female gametophyte, and it has therefore gone on in the course of evolution much as other conifers have done.

The persistence of the male cytoplasm in the egg has now been recorded for a number of genera of the Araucarineae, Podocarpineae, Taxodineae, and Cupressineae, but I have seen no record of it among the Abietineae. The majority of these records relate to genera (*Agathis*, *Phyllocladus*, *Podocarpus*, *Torreya*, and *Cephalo-*

taxus) that have suggestive resemblances in other respects to one another.

EAMES (8) has laid special stress on the fact that in *Agathis* the fusion nucleus remains in the center of the archegonium and that its divisions are limited to a restricted part of the egg cytoplasm. He looks on this as a specialization. I should be inclined to minimize the importance of this feature, for in *Araucaria* division occurs wherever the impact of the male cells has left the egg nucleus. Neither is the restriction a noticeable feature further than is determined by the fact that the proembryonic free nuclei are restricted to the limits of the male cytoplasm that envelops them.

The irregular division in the proembryo, the indefinite number of nuclei formed, and the method of their arrangement distinguish *Araucaria* rather sharply from the Abietineae, though some or all of these features are paralleled among the other tribes.

The number of cells in the proembryo before elongation, and the time of wall formation vary widely. Abietineae usually, at least, have four tiers of four cells each. In the Cupressineae the cells are usually fewer and not so regularly arranged. Among the Taxaceae the numbers run much higher (18–32) and the arrangement is still less regular. Walls form somewhere about the 8-celled stage in Pinaceae, but at widely different stages among the Taxaceae.

In all other recorded conifers, excepting *Actinostrobus* (**18a**), the proembryo is arranged in more or less regular vertical tiers and the growth is downward. In the araucarians the embryo is not tiered, but concentric at the time walls are formed. It takes on a pseudotiered appearance later through the elongation of the upper cells of the concentric outer layer to form the suspensor and the lower ones to form a cap. In this respect the proembyro is unique, though *Cephalotaxus* (**12b, 23**), *Sciadopitys* (**17**), and some species of *Podocarpus* (**21**) resemble it in having a cap below the embryo cells. In neither of these, however, is the embryo group completely surrounded as in the araucarians. These resemblances do not contradict a relationship between araucarians and taxads, nor do they add very much strength to the evidence for it. The structure of the proembryo is so different from that of the Abietineae that it is not easy to see how it could have been developed from it.

Notwithstanding the opinions (**8, 23**) expressed by other authors as to the specialization of the proembryo of the araucarians, I am inclined to think that too much stress has been placed on the appearance it presents after elongation has begun. At that time the very definite cap, the suspensors, and the inclosed embryo group give it an appearance of specialization that is not representative of its method of development. As I have pointed out in a preceding paragraph, this very definite structure arises from a group of free nuclei that do nothing in a definite and regular fashion. The number of nuclei is indefinite, the arrangement is that of an irregular mass, the order of wall formation varies, in short nothing is definite or fixed except that the upper and lower nuclei of the mass will elongate and result in the production of a proembryo in which the position and function of the various cells appear to have been planned with the greatest care. The course of development is, in fact, far less regular and definite than that of the Abietineae, though the result is far more striking.

The formation of a secondary suspensor region from the base of the mass of cells developed from the embryo group is a feature that has not been recorded for other conifers, so far as I have been able to discover from the literature available. The nearest approach to it is in *Torreya taxifolia* (**6**), another taxad, where there is said to be a wave of elongation beginning with the second tier and involving the successive tiers downward until finally cells formed from the embryo groups are involved. It is not unlikely that this feature may be found less rare than the records at present indicate, for our knowledge of the later development of the embryo is still very meager in conifers generally.

Conclusions

1. The structure and development of the pollen tube, processes of fertilization, and the structure and development of the embryo are such that it seems extremely improbable that they could have been derived from the analogous structures as represented in modern Abietineae.

2. The structure of the seed and pollination apparatus of the araucarians could be readily derived from the type of seeds or ovules represented by such lycopods as *Miadesmia*.

3. There is some reason to suppose that some of the Cordaitales may have had ovules of the same general type as the lycopods just mentioned. If so, they were probably pollinated on the scale and might have given rise to modern conifers.

4. It would be possible to derive modern conifers from a mesozoic stock which had ovules and pollination apparatus comparable to that now possessed by the araucarians.

Summary

1. Pollination occurs on the scale at a distance from the nucellus.

2. The pollen tube is very long and gives rise to many small lateral haustorial branches. It combines features of conifers and cycads to a certain extent.

3. Reasons are adduced to show that this is probably an extremely primitive form of tube, having come down from very remote times little changed.

4. The body cell divides in the extra-nucellar part of the tube a month or more before fertilization. The central cells of the archegonium divides very late or perhaps not at all, except in cases of delayed fertilization.

5. The male cells are very large and unusually active, as well as long-lived.

6. Blepharoplast-like bodies are found in the male cytoplasm.

7. The male cells pass through the neck without injuring the cells.

8. The male cell comes into violent contact with the egg and frequently displaces it.

9. The free nuclear divisions of the proembryo are restricted to the male cytoplasm that surrounds the fusion nucleus, which persists and grows with the proembryo.

10. The male cytoplasm around the older proembryo may be surrounded by a membrane.

11. The number of free nuclei in the embryo varies from 32 to 45 or perhaps more.

12. When walls form the free nuclei are arranged concentrically.

13. The upper peripheral nuclei form the suspensor, the lower ones the cap, and the middle girdle elongates to unite cap and suspensor.

14. The central cells of the proembryo alone take part in forming the embryo.

15. In the growth of the embryonic group the cap is thrust aside and a cylinder of meristematic tissue is organized.

16. The upper portion of the embryonic cylinder functions as a secondary suspensor.

17. The definitive embryo is organized out of a portion of the cells arising from the development of the embryo group of the proembryo.

18. It is dicotyledonous, has resin ducts in the cortex but not in the wood, and is stored full of food materials (large proteid granules and smaller starch grains).

19. The cells of the prothallus become very large and crowded with food.

20. The nucellus persists and becomes a part of the testa of the seed.

21. The embryo continues intraseminal growth after the seeds are shed.

22. It is concluded that, so far as the pollination apparatus and seed structure are concerned, the Araucarineae could be derived from the lycopods, or perhaps from the Cordaitales, but not from the Abietineae. The latter might be derived from a primitive mesozoic stock resembling the araucarians in respect to these features.

STANFORD UNIVERSITY
CALIFORNIA

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EXPLANATION OF PLATES I–III

FIG. 1.—A pollen tube branching after entering the nucellus; the tip of the tube is just at the upper border of the figure.

FIG. 2.—A part of the body cell, showing part of the upper male nucleus and what appears to be the remains of the nuclear spindle concerned in the division of the body cell nucleus; the male nuclei are in the resting state, but the cytoplasm has not yet begun to divide.

FIG. 3.—The two male nuclei in a body cell whose cytoplasm has not yet completely separated into distinct male cells; $\times 250$.

FIG. 4.—Two male nuclei in a pollen tube running horizontally between the nucellar cap and the female gametophyte; the cytoplasm is unusually scanty and shows no sign of division, nor does either nucleus appear larger or more active than the other; $\times 250$.

FIG. 5.—A binucleate body cell rounding a sharp corner of the pollen tube where it turns back from the surface of the scale to enter the nucellus; a part of the pollen tube wall is shown above and another part in the bend on the right; $\times 250$.

FIG. 6.—A fully formed male cell or sperm about half-way down the pollen tube; the nucleus is at the forward end and a fragment of the degenerating nucleus that should have formed a separate male cell out of the part of the cytoplasm above and to the left of the cleavage furrow; $\times 250$.

FIG. 7.—The egg nucleus and disintegrating ventral canal nucleus; $\times 250$.

FIG. 8.—Upper end of an archegonium with a large ventral canal nucleus (at the top), a small egg nucleus (bottom), and a small extra nucleus between; $\times 250$.

FIG. 9.—A mature egg nucleus showing the dense chromatin masses distributed on the delicate linin network and to a less extent in contact with the nuclear membrane; the wrinkles in the nuclear membrane are doubtless due to the effects of the reagents used in preparation; $\times 560$.

FIG. 10.—The neck of an archegonium through which a pair of male cells has entered; note that the neck has not been ruptured, though the passage is

very much smaller than the diameter of a male cell; the figure also shows how the egg cytoplasm has been crowded out through the neck by the entrance of the male cells; ×250.

FIG. 11.—Fertilization: the male nucleus is above; both nuclei are enveloped already in the male cytoplasm, which is distinguished from the egg cytoplasm around it by being much denser; this figure shows the only case observed in which the male nucleus is larger than the female; ×250.

FIGS. 12 and 13.—Two consecutive sections through a male and female nucleus in the act of fusing; the male nucleus is to the left of the figure; the nuclear membrane has broken down in the middle region of contact (fig. 13), but not throughout (fig. 12); each nucleus is enveloped in a distinct sheath of cytoplasm, probably derived from the kinoplasmic layer sometimes surrounding the egg nucleus, as well as from the male cytoplasm; ×250.

FIG. 14.—A median section through two nuclei in which the nuclear membrane had not yet broken down, showing how the male nucleus flattens out and applies itself to the curved surface of the egg nucleus; ×250.

FIG. 15.—Fusion of two nuclei showing the fine-grained nuclear contents and the weakening of the nuclear membranes; two blepharoplast-like bodies are also shown; the left-hand one may be, possibly, a vegetative nucleus in an advanced stage of degeneration; ×250.

FIG. 16.—The second male cell in an archegonium: the male cell is cut in the median plane, but only one of the two nuclei of the proembryo below is shown; the dark portion in the center is the nucleus crowded full of large masses of chromatin-like material; around it is seen the zone of male cytoplasm appearing lighter than the surrounding egg cytoplasm or the inclosed nucleus; this cell has probably become considerably changed through degeneration; ×250.

FIG. 17.—Another section through the same archegonium as the preceding, showing one of the blepharoplast-like bodies in the edge of the male cytoplasm and a nearly median section of the 2-celled proembryo; ×250.

FIG. 18.—Median section of a 2-celled proembryo; ×250.

FIG. 19.—Another 2-celled proembryo; ×250; figs. 17–19 show that the first division may occur in any plane, horizontal, vertical, or oblique.

FIG. 20.—Median section of a 4-nucleate proembryo; ×250.

FIG. 21.—A 6-nucleate proembryo; ×250.

FIG. 22.—A 5-nucleate proembryo; ×250.

FIG. 23.—A 4-nucleate proembryo with all the nuclei in nearly the same vertical plane and at the bottom of the cytoplasm; ×250.

FIG. 24.—A 9-nucleate proembryo; ×250.

FIGS. 25–29.—Proembryos with 15–40 nuclei variously arranged, but none with exactly 16 or 32; ×250.

FIG. 30.—A 45-nucleate proembryo, with the nuclei properly arranged for wall formation; the cap nuclei are already beginning to elongate; ×250.

FIG. 31.—The formation of walls and elongation of suspensors before the cap cells have begun to elongate or have completed wall formation; ×250.

FIG. 32.—Wall formation and elongation in the cap cells before either elongation or wall formation has begun in the suspensors; a small part of the membrane that sometimes forms above or even around the proembryo is shown at the upper right of the figure; ×250.

FIGS. 33 and 34.—A proembryo cut full length, showing all its parts after the elongation of the suspensors has begun; fig. 33 shows the expanded top of the suspensors crowded up against the neck of the archegonium; the archegonium jacket membrane is much stretched but has not broken nor has the neck been ruptured; fig. 34 shows the suspensors and the progress of destruction of the cells of the gametophyte; ×62.

FIG. 35.—Tip of a proembryo, showing the bottom of the suspensors, the group of embryo-forming cells, and the cap; note that the embryo is not properly a tiered one, and that the cell contents of the three regions are exceedingly similar; the walls of the cap cells are also seen not to be specially thicker or otherwise prepared for mechanical penetration; ×250.

FIG. 36.—Three proembryos in competition for the favored position in the endosperm; the multinucleate condition of some of the endosperm cells is also shown; the fine grains in these cells are starch and the large light colored patches are vacuoles; ×62.

FIG. 37.—The struggle for supremacy during which the proembryos coil around one another and greatly erode the gametophyte; ×20.

FIG. 38.—The beginnings of growth in the embryonic group of cells; the suspensor cells lose their cytoplasm and become distended, and the cap cells shrink and degenerate; ×175.

FIG. 39.—Further growth in the embryo group: the cap has been crushed and is being thrust to one side; cell division is more rapid in the tip region of the future embryo, while the upper cells are beginning to elongate, foreshadowing the production in that region of the secondary suspensor; a few cells of the primary suspensor are shown at the top; ×250.

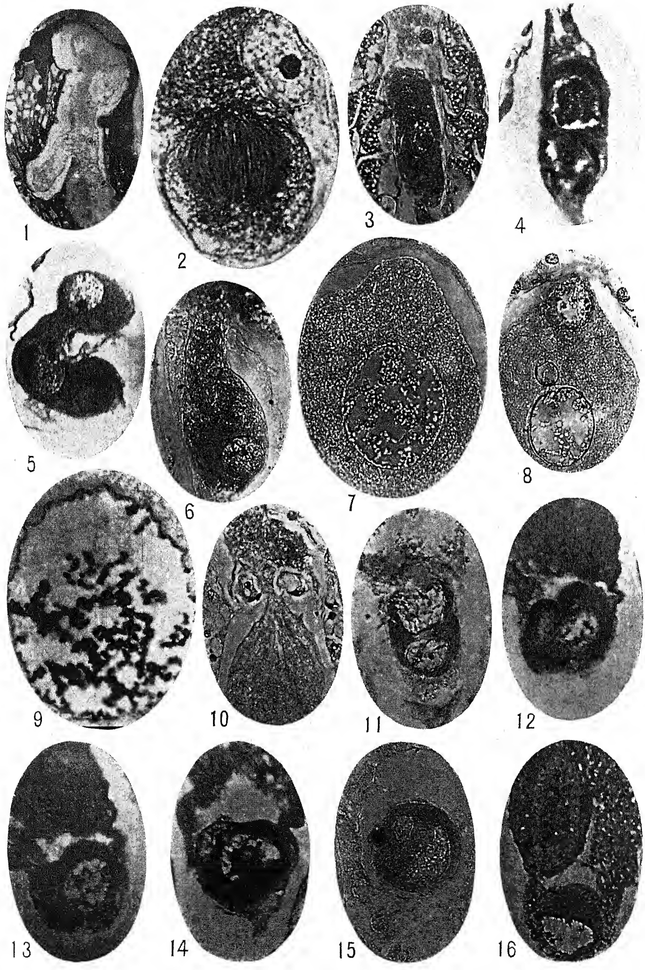
FIG. 40.—The secondary suspensors pushing the meristematic apex deep into the endosperm; note the massive character of the secondary suspensor when compared with the slender primary one; ×62.

FIG. 41.—A later stage when the meristematic region has become large; the differentiation of the body regions shown in the next figure will follow shortly after the stage shown in this figure; ×20.

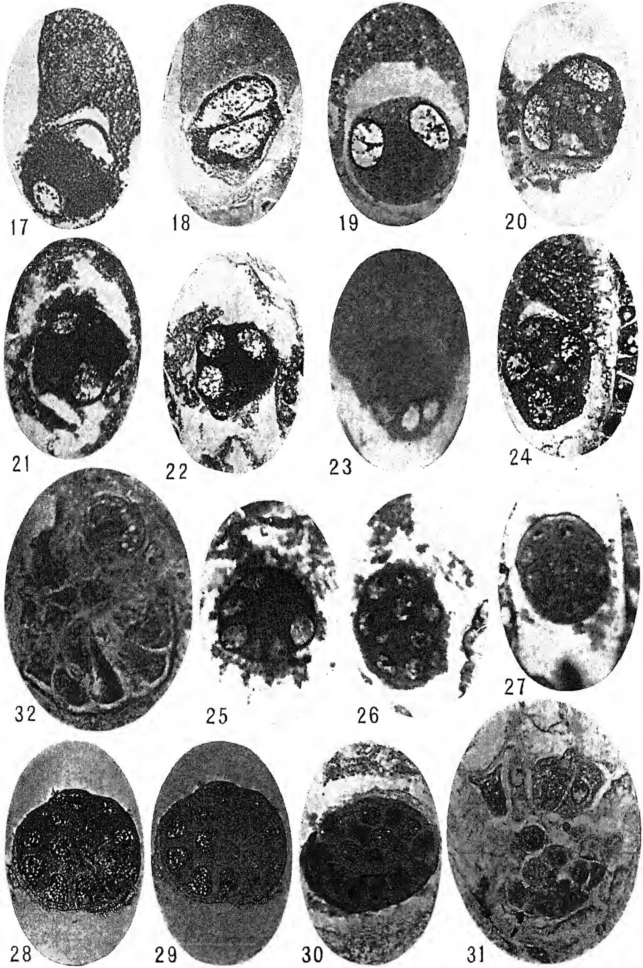
FIG. 42.—An embryo just after all the main body regions have been differentiated; ×7.

FIG. 43.—Transverse section through the hypocotyl of a nearly mature embryo, showing the vascular ring, resin ducts, and cells crowded full of starch and proteids; ×15.

FIG. 44.—Section through the cotyledons; ×15.



BURLINGAME on ARAUCARIA



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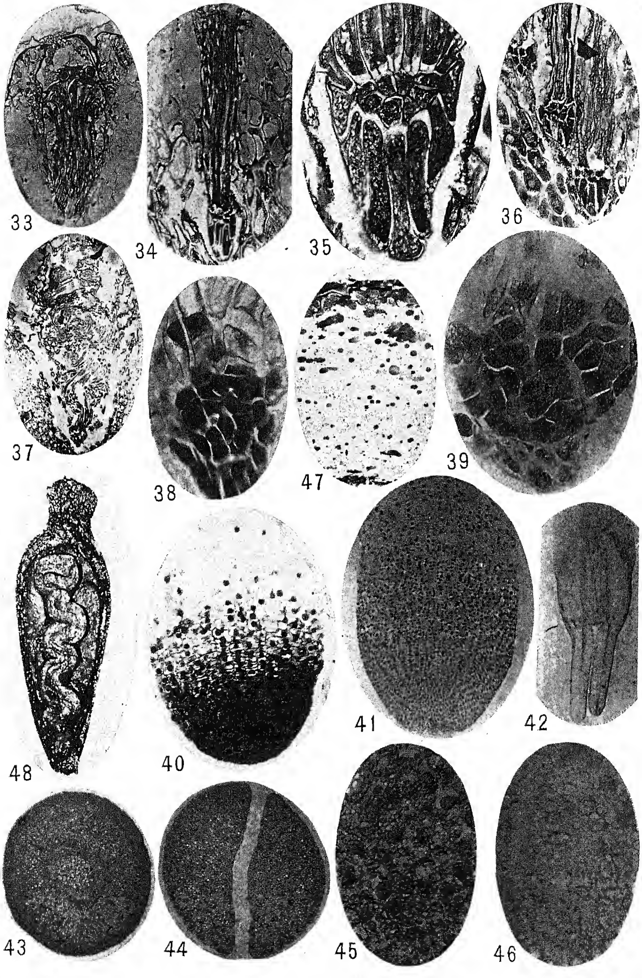
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FIG. 45.—Cortical cells from the embryo filled with small round starch grains and large proteid globules; ×250.

FIG. 46.—A single cell from the middle region of the endosperm adjacent to the embryo, showing the large oval starch grains and the tiny round proteid globules; the light lines mark the position of the delicate walls which do not show in the photograph owing to their not having been stained; ×250.

FIG. 47.—Section through the integument, showing the three layers; the central irregular cells later become woody to form the firm brown testa of the mature seed.

FIG. 48.—Longitudinal section of a seed that had been kept for over a year in a tin box in laboratory; it shows how growth of the embryo continues after the fall of the seed, even under comparatively unfavorable conditions; natural size.

THE ARCHEGONIUM OF SPHAGNUM SUBSECUNDUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 199

George S. Bryan

(WITH PLATES IV–VII)

This paper is planned as the first of a series on the life history of *Sphagnum subsecundum*, the work being undertaken through interest in the Sphagnales aroused during a course in the special morphology of Bryophytes under Dr. W. J. G. Land at the University of Chicago. It is hoped that this investigation may not only bring to light new facts in the life history of *Sphagnum*, but when completed may enable us to determine more accurately the position of the Sphagnales in the phylogeny of the Bryophytes.

Field study

The first stage in this undertaking, that is, securing material for study, has been something of a problem in itself. The impression prevails, in some quarters at least, that *Sphagnum* seldom bears sex organs. For example, Campbell (1, pp. 163–164) in a general discussion of the Musci says: "When the plants are dioecious it sometimes happens that the two sexes do not grow near together, in which case, although archegonia may be plentiful they fail to be fecundated and then no capsules are developed. This no doubt accounts for the extreme rarity of the sporogonium in many mosses, although in other cases, e.g., *Sphagnum*, it would appear that the formation of sex organs is a rare occurrence."

On the other hand, those investigators who have made studies of the sex organs of *Sphagnum* seen to have had little difficulty in securing material. Leitgeb (8) alone reports his study of the archegonium hampered by lack of material.

Again, there is disagreement as to whether or not the archegonial branches have characters by which they may be distinguished. As is well known, antheridial branches on approaching maturity are marked by their coloration. Have the archegonial branches such a well defined character? Campbell (1, p. 177), referring

to the Sphagnales, says: "The archegonia are found at the apex of some of the short branches at the summit of the plant, which externally are indistinguishable from the sterile branches." CAVERS (2, p. 295), on the contrary, says: "The female branch grows for some time in the same way as an ordinary sterile branch, but the leaves visible from the outside of the branch become rapidly longer in passing from below upward, so that the branch takes the form of a loose, tapering, pointed bud, deep green in color, and stands out sharply from the vegetative branches associated with it."

Furthermore, there is some disagreement as to the time of the appearance of sex organs. According to SCHIMPER (10), antheridia are produced at any time, but are most abundant in autumn and winter. WALDNER (11) found mature antheridia and archegonia under the snow in February. GAYET (3) reports having spent much time searching for archegonia in the winter, but only in the spring did he find them developing. However, he emphasizes the point that he does not wish to say archegonia are not produced at all in the winter, but rather that it is only in the early spring time one finds them being formed in the greatest abundance.

To summarize, we find: (1) sex organs are believed by some to be of rare occurrence; (2) there is disagreement as to the recognition of archegonial branches; (3) there is some uncertainty concerning the time of the production of sex organs.

To investigate these points in regard to *S. subsecundum* we have made a careful study of a bog covering about 20 acres near Mineral Springs, Indiana, 40 miles south of Chicago. This place was selected for several reasons. It contains enough water in the spring to escape being burned over, the annual fate of many bogs in the vicinity of Chicago, hence the study could be carried on without fear of having the material injured by fire. Also in previous years some sporophytes had been noted here. Furthermore, polsters of a *Sphagnum* (later identified by Mr. E. J. HILL as *S. subsecundum*) were well scattered through the bog, affording a wealth of material.

Active work was begun the first week in November 1912. It soon became evident that *S. subsecundum* here studied is dioecious, and that when the sex organs are approaching maturity both

male and female plants have well marked characters. The antheridial heads are decidedly globose and show variations in color from yellow brown to red brown, occasionally almost black. The archegonial heads are less globose, have a somewhat flattened aspect on top, and show no unusual coloring except the conspicuous bud at the growing point in the center of the head. This bud varies in color from yellow brown to red brown. An analysis of the bud reveals archegonia almost mature on short side branches near the apex of the main axis, the coloring matter being in the perichaetial leaves surrounding the organs.

A careful study of the whole bog and others for several miles about disclosed the fact that not a single sterile head of *S. subsecundum* could be found. Sex organs were everywhere in vast numbers. In order to determine whether or not such a condition might be unusual, and to provide abundant material for study, developments through winter, spring, summer, and autumn of 1913 were closely followed; and again in the autumn of 1913 the sex organs appeared in the same vast numbers.

As far as *S. subsecundum* in the vicinity of Mineral Springs is concerned, we conclude therefore (1) that sex organs are not of rare occurrence; (2) that both antheridial and archegonial heads on approaching maturity are distinctly characterized by coloration; (3) that antheridia and archegonia begin to develop in August and September respectively, the antheridia always appearing first.

A brief statement of time relations in the developmental process of the archegonium as observed in the autumn of 1913 may be of interest. Young stages were first noted on September 16 and continued to appear for approximately four weeks. By October 25 the youngest archegonia were beginning the formation of canal cells, while the oldest were almost mature. At this time the coloring of the perichaetial leaves began to be noticeable. By November 15 the canal row had broken down in some of the oldest archegonia. Those archegonia which have not reached maturity on the advent of cold weather develop slowly through the winter. In the spring, therefore, at the time of the disappearance of the snow, it is possible to find stages having 7 or 8 canal cells, with the ventral cell not yet divided.

Methods

For this investigation almost daily killings were made, from the middle of September to the middle of November. The killing agent used was 0.25 chrom-acetic, cold, and heated to various temperatures up to 52° C., the best results obtained being at about 30° C. At this temperature there was practically no plasmolysis in the delicate young stages, and the quick penetration made certain the securing of any figures that might be present in the material. The higher temperatures were not satisfactory, causing more or less plasmolysis and leaving the material difficult to stain. Safranin in combination with Licht Grün, and Haidenhain's iron alum hematoxylin were used as stains.

Development of the archegonium

HISTORICAL

The archegonium of *Sphagnum* has been the subject of a number of investigations. Of the early papers the most important is the elaborate monograph of SCHIMPER (10). According to this investigator the archegonium, arising directly from the apical cell of a branch, begins its development by an apical cell with two cutting faces, just as in the true mosses, and forms in this manner about 6 cells. The events that follow, being beyond the technique of that time, are described in a hazy manner and may be passed over here. Paraphyses are said to occur among the archegonia.

The brief account given by HOFMEISTER (5) does not differ from that of SCHIMPER.

In 1869 LEITGEB (8), while working out the development of the antheridium, found a few female branches, and on each, one archegonium was being formed, arising directly from the apical cell of the branch, but whether the divergence of the division walls is one-half, as HOFMEISTER and SCHIMPER thought, or whether, as in the antheridium, there are smaller divergences, and furthermore from what cells the secondary archegonia arise, he is unable to say because of a lack of material.

The account given by JANCZEWSKI (7) in 1872 may be summarized as follows. The apical cell elongates and is divided by

cross-walls, finally consisting of 3 or 4 cylindrical cells, each of which makes secondary divisions, and above these two cells whose walls are obliquely placed and which also make secondary divisions. The origin of the archegonium proper and the formation of adventitious segments and canal initials are declared to be the same as among the mosses. In *Sphagnum rigidum* and *S. acutifolium* "anomalies" in the development are reported, though the regular process described above also occurs. No explanation is offered as to what these "anomalies" are. It is to be regretted that no illustrations accompany the article.

The most recent account is that given by GAYET (3) in 1897. He agrees with LEITGEB that the first archegonium is axillary, but dismisses the early stages with the brief statement that "the first divisions are normal." The two figures given to illustrate this are by no means clear, so that one is left in doubt as to the meaning of the word normal. GAYET is unable to find the two cells with oblique walls reported by JANCZEWSKI and thinks them an error of interpretation. The neck of the archegonium is said to elongate by the division of the terminal cell, and this terminal growth is produced without giving rise to canal cells.

From these brief reviews it is evident that there is little agreement among investigators as to developmental processes, and that the whole subject is in a haze of uncertainty.

EARLY STAGES IN THE DEVELOPMENT OF THE ARCHEGONIUM

In the autumn, at the time of the production of sex organs, the elongation of the main axis is checked, so that the newly formed branches whose apical cells are being transformed into archegonia appear as a cluster or bud about the main axis at the apex. This transformation of the apical cells of the side branches into archegonia is not simultaneous, but proceeds acropetally, occurring earlier and earlier in the development of each branch as one passes toward the apex. As yet this transformation process has not been observed to reach the apical cell of the main stem, though more than 400 slides bearing on this point have been examined.

In the material studied the maximum number of archegonia arising from an apical cell is three. In such a case each of the

two segments last formed becomes the initial of a secondary archegonium, while that portion of the apical cell above and not included by them is the initial of the primary archegonium (fig. 2). Fig. 3 shows this in cross-section. A few examples have been noted where one of the secondary segments, after making several divisions, has for some reason been checked and remains as a slight projection on the base of the mature primary archegonium. In some cases only the last formed segment develops as a secondary archegonium; while still more rarely no secondary archegonia are formed at all, the apical cell becoming the initial of a single archegonium (fig. 5).

THE PRIMARY ARCHEGONIUM

The primary archegonium shows a remarkable variation in the manner of its early divisions. The first wall may be transverse (fig. 6) or slanting (fig. 4). If the first wall is slanting, the second may be transverse (fig. 5). However, by the examination of a large number of slides one may recognize two general types of development. A filament of cells, usually 4 or 5 in number, may be formed by successive transverse divisions of the apical cell (figs. 6–9). Four or five cells may be produced by the activity of an apical cell with two cutting faces (figs. 11, 12); this is probably the most frequent method. Between these two extremes there may be various mixtures of planes. An interesting intermediate condition in which the walls do not quite intersect is shown in figs. 10 and 13.

At this point the question may be asked, Why are not figs. 6–9 merely the development of an apical cell with two cutting faces seen at an angle of 90° from the plane represented by figs. 11 and 12? This matter has been carefully examined and the following facts presented as an answer. Unquestionably the walls may have such an appearance, but the test is their behavior under the oil immersion lens. If the walls are transverse, they remain steady on focusing up and down; but if of the kind formed by an apical cell with two cutting faces, they swing in a characteristic manner as one changes the focus. Frequent examples of this have been found, as well as those in which there was no shifting.

Hence figs. 6–9, belonging to this latter class, are known to be transverse.

THE SECONDARY ARCHEGONIUM

The secondary archegonium shows a greater degree of uniformity. The initial divides into an inner and an outer cell (figs. 6, 10). This outer cell by subsequent transverse divisions (figs. 8, 9, 14) gives rise to a filament of cells, 5 or 6 in number, in each of which the usual secondary divisions occur (fig. 16*b*). As yet no evidence has been found that the secondary archegonium may develop by an apical cell with two cutting faces.

THE DEVELOPMENT OF THE ARCHEGONIUM PROPER

After there has been formed, as described above, a filament of cells by transverse walls, or a series of cells by an apical cell with two cutting faces, and secondary divisions have occurred in each segment except the terminal one, the development of the archegonium proper begins in the manner usual among the Bryophytes. In the terminal cell, which becomes somewhat enlarged, three oblique walls appear, cutting off three peripheral segments and originating a large cell within, which has the form of an inverted truncated pyramid (figs. 13, 14). This large cell we shall designate the primary axial cell. On division it gives rise to an outer axial cell, the cover cell, and an inner axial cell, the central cell (figs. 17–19). The wall cells of the archegonium arise from the three peripheral segments.

Up to this point the development of the archegonium proper coincides exactly with the description given by numerous investigators for the archegonium of the Bryophytes, whether Hepaticae or Musci. It is in the events immediately following that there is a divergence of views and theories. For the sake of continuity we shall postpone the presentation of these theories until later and continue the description of the developmental processes.

Here then the important question arises, What is the part played by the cover cell and what by the central cell in the further development of the archegonium? The answer must be found in a study of sections both longitudinal and transverse.

THE COVER CELL

Figs. 18 and 19 represent typical cases in the appearance of the archegonium with cover cell and central cell formed. It will be noted that the two cover cells differ in size. This is due to the peculiar shape of the cover cell, coupled with the direction of the cut. In fig. 18 the cut is median through the long diameter; in fig. 19 through a shorter diameter. This may be more clearly understood by an examination of the cover cells as shown in transverse sections (figs. 53–55).

It is important to note that the cover cell may divide by a vertical wall into two almost equal segments before the division of the central cell takes place (figs. 21, 54). But more important still is the evidence that by the time the central cell has completed its division into primary neck canal cell and primary ventral cell, the cover cell has at least become divided by a vertical wall into two almost equal segments (figs. 22, 24), and in some cases has formed a quadrant of cells (figs. 23, 56). The division lines between the cells of the cover and the outer cells of the neck are clearly marked and easy to follow in the younger stages. Thus in figs. 29–32 the cover is literally the cap of the archegonium, and in each case contains 6 cells (three each in median longitudinal section, as illustrated). In fig. 33 the cover consists of 8 cells. However, in the older stages the cells of the cover and the neck usually merge so insensibly that the two cannot be separated with any degree of certainty. Hence no accurate statement as to the final number of cells produced by the cover can be made.

From the foregoing facts it is evident that the cover cell divides early by a vertical wall into two almost equal segments. Subsequent vertical divisions in each of these segments produce a plate of cells, 8 or more in number, which covers the apex of the archegonium and in mature forms merges insensibly with the upper cells of the neck. There is not the slightest evidence to show that the cover cell cuts off any basal segments.

THE CENTRAL CELL

The division of the central cell into primary neck canal cell and primary ventral cell is shown in figs. 20 and 21. The primary

neck canal cell is the mother cell of the neck canal row. The spindle for the division into two canal cells, as shown in fig. 25, has been found four times in the material studied. From this point on the cells of the canal row divide in almost any order. The evidence for this shown by the spindles in figs. 27, 28, 30, 31, and 34. By this intercalary growth a row of canal cells, usually 8 or 9 in number, is formed (figs. 38, 40).

The division of the primary ventral cell occurs late. Fig. 38 shows 8 canal cells and the ventral cell undivided; while we were fortunate enough to find a spindle when there were 7 canal cells (fig. 39). The ventral canal nucleus produced by this division is peculiar, being only a trifle smaller than the egg, and is remarkable in that it is regularly persistent and behaves for a time just as does the egg. Not long after the division into ventral canal cell and egg, the canal row begins to disintegrate (this process having a variable beginning, through quite often acropetal), but not so the ventral canal cell. Its cytoplasm begins to condense about the nucleus (the same process occurring about the egg), and soon we have in a mature archegonium the appearance of two eggs separated by a wall (fig. 41). Later the cytoplasm about each of these two nuclei becomes markedly condensed and rounded off and may be easily observed in the living material. Still later the wall between the two cells breaks down and the nuclei, each as the center of a ball of cytoplasm, come to lie near together in the venter of the archegonium. Usually just before fertilization the ventral canal nucleus disintegrates.

THE GROWTH OF THE ARCHEGONIUM

We have already shown that the growth of the canal row is intercalary. The same is true of the growth of the wall cells. Fig. 26 gives valuable evidence on this point. It is not an exceptional case, but was found a number of times. The evidence goes to show that about the stage of two canal cells there comes a sudden vigorous growth of the archegonium through intercalary divisions, this process frequently involving one or two rows of cells simultaneously. This sometimes results in one side of the archegonium becoming longer than the other, tilting the cover, as is shown in

figs. 33–35. In the older stages the growth is slower, but spindles in various cells of the periphery give further undoubted evidence of intercalary growth.

THE MATURE ARCHEGONIUM

In the account of the development of the archegonium given by the various authors already mentioned there has been much discussion concerning the mature archegonium. It will be of interest, therefore, to record the facts in regard to *S. subsecundum*. The archegonia here may be divided into two classes or types, those long and slender and those massive. This difference begins to appear early and may be clearly seen by a comparison of figs. 30 and 32 or 34 and 35. Naturally, therefore, we find variability in the mature stages. In its simplest portion, the neck for a short distance may have 6 rows of cells, or in the more massive types each cell of the 6 rows may have one or more secondary divisions. Fig. 58 represents a typical series through the simplest portion of such a neck. The neck merges gradually into the venter, which is usually 4 cells thick (fig. 59), though simpler venters may also be found.

ABNORMALITIES

Abnormalities are of rather frequent occurrence in *S. subsecundum.* Double venters (fig. 42), unequal division of the venter, the ventral canal nucleus larger than the egg (fig. 43), ventral canal nucleus the same size as the egg (fig. 44), and multiple eggs (fig. 45) are not of rare occurrence.

THE ABSENCE OF PARAPHYSES

Schimper (10) in his elaborate monograph reports structures among both antheridia and archegonia which he calls paraphyses. Other investigators have been unable to find any trace of paraphyses. We have taken particular pains to investigate this in *S. subsecundum*, dissecting hundreds of heads, both antheridial and archegonial, but not the slightest indications of paraphyses could be found. This was further confirmed by an examination of about 500 slides with the same result. In a few cases the branched hyphae of a fungus, *Tilletia Sphagni*, were observed

about some of the archegonia. This, as has already been suggested by several investigators, may account for the so-called paraphyses of SCHIMPER. We feel safe, therefore, in stating that so far as *S. subsecundum* is concerned there are no paraphyses about either the antheridia or the archegonia.

THE MUCILAGE HAIRS

The peculiar structures developing in the axil of each young leaf have been commented on by several investigators. A complete series in the development may be easily followed out. One of the axillary cells at the base of the leaf becomes papillate (fig. 47), divides into an upper cell and a basal cell (fig. 49), and the upper cell makes two acropetal divisions resulting in a filament of three cells (figs. 49, 50). This is the mature stage. The terminal cell of the filament usually becomes enlarged and is filled with a dark staining substance, probably mucilage. Several mucilage hairs may arise from the axillary row at the base of a leaf (fig. 49). Occasionally branched forms may be found (fig. 51). As the leaf grows older the hairs disappear.

Discussion

So far as the young stages in the development on the archegonium are concerned, it appears that HOFMEISTER (5), SCHIMPER (10), and JANCZEWSKI (7) are all correct. An examination of many sections shows development by all the methods reported, as well as intermediate conditions not reported. In the development of the archegonium proper we are unable to find any evidence to support the statement of JANCZEWSKI (7) that adventitious segments and canal initials are cut off as in the Musci. Furthermore, the evidence is clear and emphatic that the growth of the archegonium is not terminal, as GAYET (3) holds, but is intercalary. The spindles shown in various figures are conclusive on this point.

We must now consider briefly the theories of archegonial development among the Bryophytes, and the natural question as to what bearing this investigation has upon these theories.

JANCZEWSKI (7), GOEBEL (4), CAMPBELL (1), HOLFERTY (6), and others hold that the archegonium of the Musci is to be distin-

guished from that of the Hepaticae by its peculiar apical growth; that in the Musci the canal cells do not arise by the activity of one mother cell as in the Hepaticae, but are produced in part by the division of the cover cell. This cover cell cuts off two sets of segments, the one being parallel to the axis of the archegonium and forming the wall cells of the neck; the other parallel to the base of the archegonium and contributing to the neck canal row.

The view presented by GAYET (3) is in the main a contradiction. He holds that in both Hepaticae and Musci the growth of the archegonium is terminal, but no internal segments are added to the canal row by the cover cell, which cuts off segments forming the wall cells of the neck.

SERVETTAZ (9, pp. 169–171) in a recent paper has advanced a new interpretation of archegonial formation. His description of the development in *Phascum cuspidatum* may be summarized as follows. The initial cell divides transversely and gives a basal cell and a superior cell. The superior cell then divides obliquely a certain number of times, from two to five; then one of the cells placed below the terminal cell divides tangentially and determines the formation of a central cell, which by basipetal divisions gives a row of 8 cells, the canal row, the ventral canal cell, and the egg. The evidence offered for these statements certainly is not convincing, and if true this origin of the central cell differentiates *Phascum* from any of the Bryophytes now known.

The evidence we have presented for *Sphagnum* has nothing in it to support the views of SERVETTAZ (9) and GAYET (3). Furthermore, it breaks the distinction between Musci and Hepaticae drawn by JANCZEWSKI (7), CAMPBELL (1), GOEBEL (4), etc. Here at least is one of the Musci in which the cover cell does not add to the canal row.

Conclusions

The archegonium of *Sphagnum subsecundum* is synthetic. The stalk, the thick venter, and the comparatively slender twisted neck are moss characters; the relatively inactive cover cell, the intercalary growth of the archegonium, and the low number of canal cells are hepatic characters as we know them today.

Summary

1. During the autumns of 1912 and 1913 sex organs have been found in vast numbers on *Sphagnum subsecundum* in the vicinity of Mineral Springs, Indiana.

2. On approaching maturity the archegonial heads may be recognized by the colored bud in the center of the head at the apex. Analysis of the bud shows terminal archegonia on short side branches.

3. The archegonia begin to develop in September.

4. The apical cell of a side branch is a primordium; each of the two segments last formed becomes the initial of a secondary archegonium, while that part of the apical cell above and not included by these segments is the initial of the primary archegonium.

5. There is great irregularity in the early stages of the development of the primary archegonium: there may be a filament of cells by the successive transverse divisions of the apical cell; or growth by an apical cell with two cutting faces; or a mixture of planes.

6. As yet the secondary archegonium has been found to develop only by the successive transverse divisions of the apical cell.

7. The archegonium proper is initiated in the manner usual among the Bryophytes. In the terminal cells three oblique walls cut off three peripheral segments and originate the primary axial cell within, which on division gives rise to cover cell and central cell.

8. The cover cell is relatively inactive and cuts off no basal segments.

9. The central cell on division forms the primary neck canal cell (the mother cell of the neck canal row) and the primary ventral cell.

10. The growth of the neck canal row is intercalary, the cells dividing in almost any order.

11. The primary ventral cell divides late into ventral canal cell and egg.

12. The growth of the wall cells of the archegonium is intercalary.

13. The mature archegonium has 8 or 9 canal cells.

14. The breaking down of the canal row may begin at any point, is frequently acropetal, but never involves the ventral canal cell.

15. The ventral canal cell is persistent, behaves for a time exactly as does the egg, but normally disintegrates just before the archegonium opens for fertilization.

16. Abnormalities, such as double venters, multiple eggs, etc., are of common occurrence.

17. The archegonium of *Sphagnum* is synthetic, combining certain characters of the Hepaticae with others of the Musci.

The author wishes to express his sincere thanks to Professor JOHN M. COULTER and Dr. W. J. G. LAND for helpful advice and discussion; and to Mr. E. J. HILL who kindly identified the material studied.

UNIVERSITY OF CHICAGO

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EXPLANATION OF PLATES IV–VII

All figures were drawn with aid of Abbé camera lucida at table level, and, being reduced one-half in reproduction, now show the following magnifications: figs. 1–35, 43–56, ×525; figs. 36–42, 57, ×300; figs. 58–59, ×180.

Abbreviations are as follows: *a*, primary archegonium or its initial; *b*, *c*, secondary archegonia or their initials; *l*, leaf.

PLATE IV

FIG. 1.—Primordium showing a primary initial and one secondary initial.

FIG. 2.—Tangential section through primordium, showing primary initial and two secondary.

FIG. 3.—Same in transverse section; dotted line shows plane of cut in fig. 2.

FIG. 4.—First wall in primary initial slanting; secondary initial has divided to form an inner and an outer cell.

FIG. 5.—First wall in archegonium initial slanting, second transverse; no secondary formed.

FIG. 6.—First wall of primary initial transverse.

FIG. 7.—Uppermost wall of primary initial transverse; next below tilts slightly; secondary not shown.

FIG. 8.—Two transverse walls in primary archegonium; secondary shows first transverse wall.

FIG. 9.—Three uppermost stories of primary archegonium formed by transverse walls.

FIG. 10.—Walls of primary archegonium do not quite intersect.

FIG. 11.—Development of the primary archegonium by an apical cell with two cutting faces; first transverse wall has appeared in the secondary archegonium.

FIG. 12.—The same, slightly older.

FIG. 13.—Stalk of archegonium formed chiefly by walls that do not intersect; in terminal story the primary axial cell has been originated by the three oblique walls.

FIG. 14.—Stalk of primary archegonium regular; primary axial cell cut out; median longitudinal section through secondary archegonium showing two transverse walls.

FIG. 15.—Tangential section through secondary archegonium showing two transverse walls.

FIG. 16.—Group consisting of primary archegonium and two secondary; the primary and one secondary shown in outline.

FIG. 17.—Division of primary axial cell into cover cell and central cell.

FIG. 18.—Cover cell and central cell.

FIG. 19.—The same.

FIG. 20.—Division of central cell into primary neck canal cell and primary ventral cell.

FIG. 21.—The same, but shows cover cell divided.

FIG. 22.—Primary neck canal cell and primary ventral cell formed; cover cell has divided into two almost equal segments; plastids beginning to be conspicuous about each nucleus.

FIG. 23.—The same, but cover is forming a quadrant; the cell on the left shows a figure, while that on the right has divided in the same plane.

PLATE V

FIG. 24.—Primary neck canal cell and primary ventral cell; unequal growth in the walls pushing cover to one side.

FIG. 25.—Primary neck canal cell in division.

FIG. 26.—Two neck canal cells and the primary ventral cell; intercalary growth in the walls of the archegonium, pushing cover to one side; plastids becoming conspicuous.

FIG. 27.—Slender type of archegonium with two neck canal cells and the primary ventral cell; the uppermost neck canal cell is in division.

FIG. 28.—Simultaneous division of the two neck canal cells.

FIG. 29.—Three neck canal cells and primary ventral cell; cover has 6 cells (three shown in median longitudinal section).

FIG. 30.—Slender type of archegonium with three neck canal cells and primary ventral cell; figure in terminal neck canal cell; cover as above.

FIG. 31.—Three neck canal cells and primary ventral cell with figure in basal neck canal cell; cover as above.

FIG. 32.—Massive type of archegonium; four neck canal cells and primary ventral cell; cover as above.

FIG. 33.—Five neck canal cells and primary ventral cell; cover, tilted to one side, has 8 cells (4 shown).

FIG. 34.—Five neck canal cells and primary ventral cell; the middle neck canal cell is in division.

FIG. 35.—Six canal cells and primary ventral cell; cover, irregular through disturbance, has 8 cells; plastids conspicuous in the canal row.

FIG. 36.—Symmetrical type of archegonium, having 6 neck canal cells and primary ventral cell; cover difficult to follow, but probably has 8 cells (4 shown).

FIG. 37.—Seven neck canal cells and primary ventral cell.

PLATE VI

FIG. 38.—Eight neck canal cells and primary ventral cell.

FIG. 39.—Seven neck canal cells and primary ventral cell in division to form ventral canal cell and egg.

FIG. 40.—Nine neck canal cells, ventral canal cell, and egg.

FIG. 41.—Neck canal cells broken down; protoplasm beginning to round off about ventral canal nucleus and egg.

FIG. 42.—Fine example of double venter.

FIG. 43.—Unequal division; ventral canal nucleus larger than egg.

FIG. 44.—Elongated venter almost equally divided; ventral canal nucleus same size as egg.

FIG. 45.—Venter with 4 cells; the 3 lowest probably are eggs, the uppermost the ventral canal cell.

FIG. 46.—Median longitudinal section through cap of mature archegonium, showing unusual divisions.

FIG. 47.—Section tangential to face of leaf; first stage in the development of mucilage hair; axillary cell at base of leaf becomes papillate.

FIG. 48.—Nucleus has divided and papillate cell cut off by a wall.

FIG. 49.—Acropetal division of the papillate cell.

FIG. 50.—Second acropetal division of the papillate cell; the mature mucilage hair.

FIG. 51.—Branched form of mucilage hair.

PLATE VII

FIG. 52.—Series of transverse sections through young primary archegonium and two secondary.

FIG. 53.—Serial sections through archegonium proper, showing cover cell and central cell.

FIG. 54.—Serial sections; the cover cell has divided, but central cell is yet undivided.

FIG. 55.—Serial sections, showing in the formation of the archegonium proper the three oblique walls which have cut off the peripheral segments and originated the primary axial cell within.

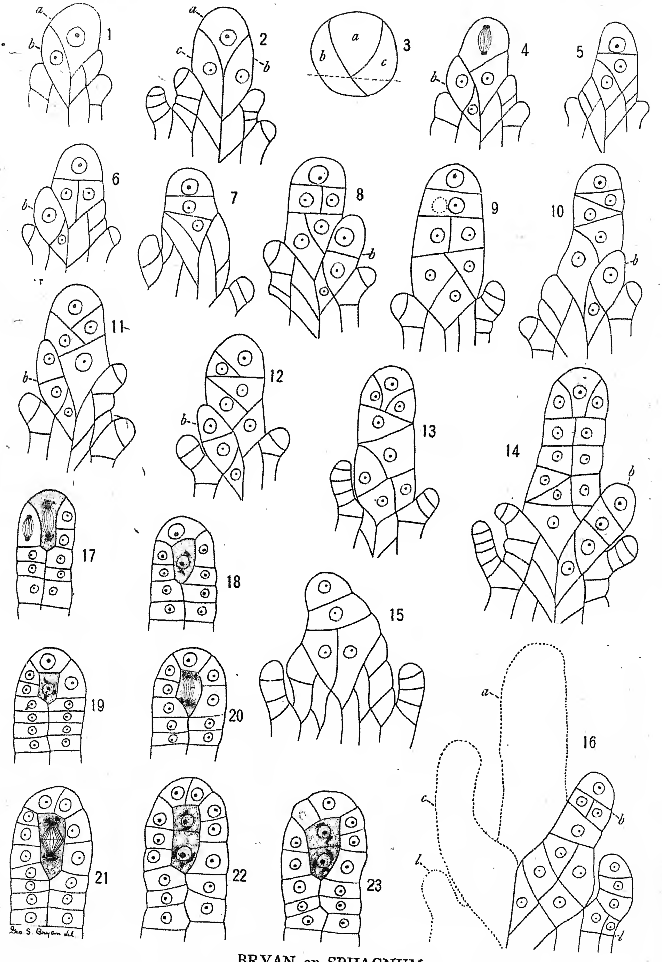
FIG. 56.—Serial sections through archegonium having primary neck canal cell and primary ventral cell; the cover cell has formed a quadrant of cells.

FIG. 57.—Serial sections through archegonium with 6 canal cells and ventral cell; the cover contains 8 cells; the gradual transition from cap to neck to venter is well shown.

FIG. 58.—Serial sections through simplest portion of neck of mature massive type of archegonium; section *A* shows the characteristic thickenings toward the cap.

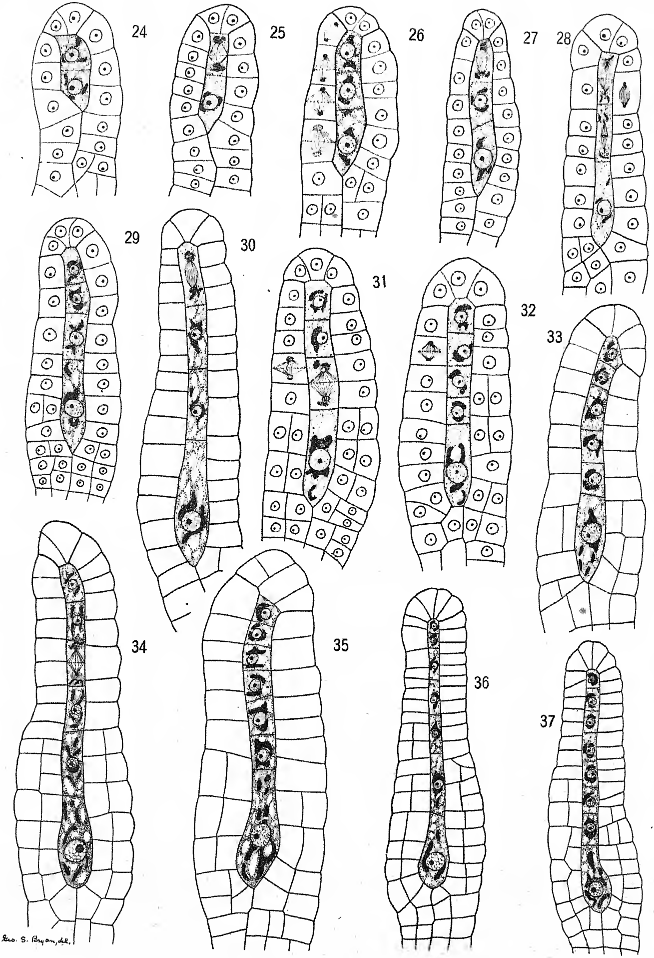
FIG. 59.—Venter of same series at level of egg nucleus.

PLATE IV



BRYAN on SPHAGNUM

PLATE V



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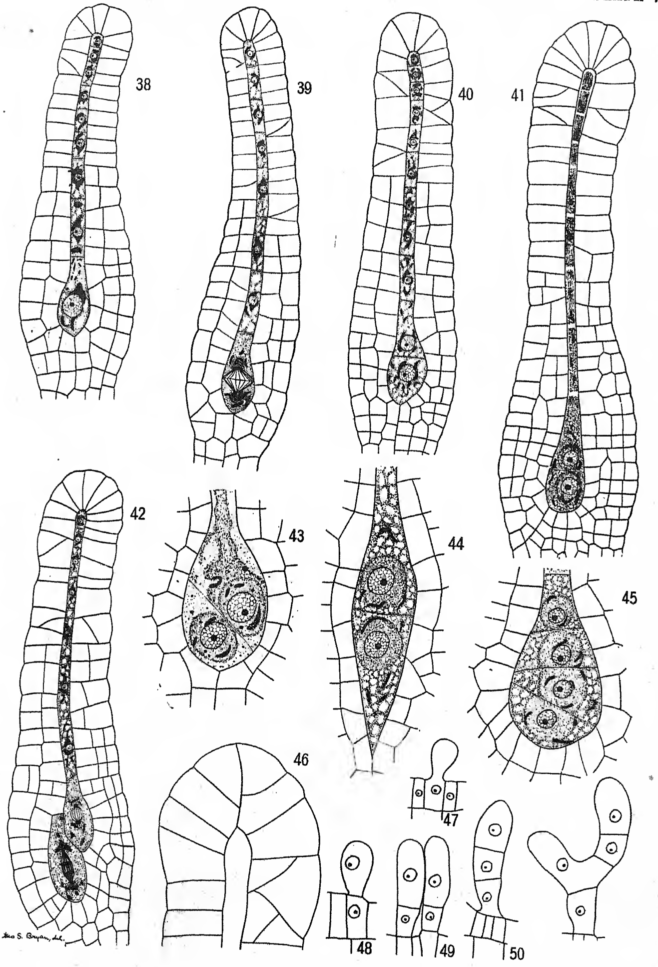

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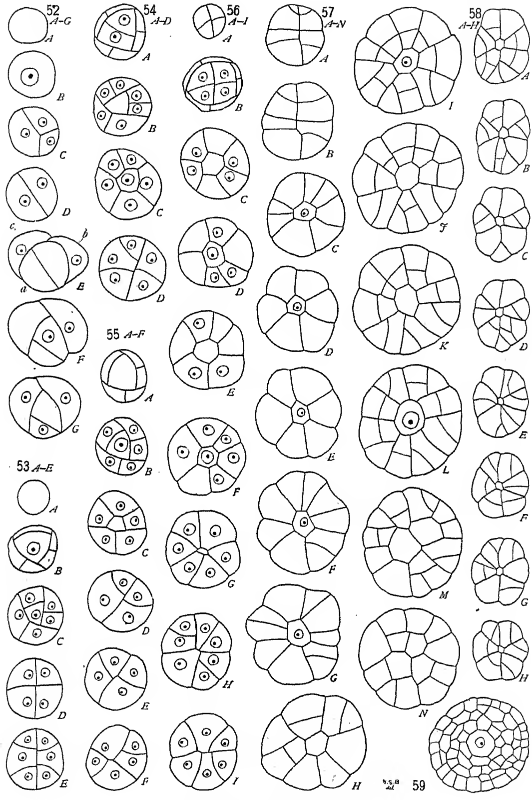

BRYAN on SPHAGNUM

CURRENT LITERATURE

BOOK REVIEWS

Plant physiology

The third German edition of JOST'S *Lectures on plant physiology*[1] shows certain changes from the first edition in general organization and point of view. Instead of the headings "Stoffwechsel," "Formwechsel," and "Energiewechsel," for the general divisions of the subject, the new edition has "Stoffwechsel," "Formwechsel," and "Ortwechsel." The heading "Energiewechsel" is used for chap. xix, the last chapter under "Stoffwechsel." This brings the energy transformations into close connection with the source of the energy, which is quite desirable in contrast with the attempt to connect it with any particular manifestation of energy, as movement. Lecture 17 of the new edition has the heading "Oxidation of hydrogen sulfid, hydrogen, methane, and ammonia by bacteria. Carbon assimilation without light and chlorophyll." It corresponds to lecture 18 of the first edition with the heading "Sulfo and nitro bacteria." Several other changes appear in lecture headings, indicative of change in viewpoint or content. The introductory lecture to "Formwechsel" has been cut down to five introductory paragraphs in the chapter on "Growth of the cell." The 43 lectures of the first edition thus become 42 in the present edition, but the pages have increased from 695 to 760.

As one reads this excellent treatise, he is impressed by the number of subjects of which the author has an excellent critical grasp; plant physiology, plant anatomy, evolution and heredity, physiological chemistry, etc. JOST has a genius for organization and is apparently a natural teacher free from pet theories or hobbies. One is inclined to compare him, as a teacher and organizer of the subject, with SACHS, remembering, however, that the task of organizing the subject is now far more difficult, owing to the enormous amount of experimentation and range of knowledge to be included. In most parts of the treatise there are excellent summaries of the subjects, entirely up to date. This is illustrated by the statement on the synthesis of amino acids, lecithin, proteins, etc., which embodies the late work of TRIER and others; by the greatly improved statement on catalysis and enzyme action; and by the lectures on hybridization and heredity, on variation, and on species-formation.

[1] JOST, LUDWIG, Vorlesungen über Pflanzenphysiologie. xvi+760. *figs. 194.* Jena: Gustav Fischer. 1913.

The reviewer believes that JOST'S treatment of transpiration could be greatly improved by adopting LIVINGSTON'S idea and method of relative transpiration. This gives quantitative determination of the inhibitory and regulating factors at work in the leaf under various conditions. The very exact work of BRIGGS and SHANTZ on wilting coefficient is passed over with mere citation, and data obtained from the rather indefinite work of SACHS on non-available water.

Many will believe that JOST'S meager and derogatory statement of the place of colloids in plant physiology is very inadequate and faces away from future progress in the subject. The statement on dormancy in seeds could have been written twenty years ago as well as now. JOST apparently attempts to illuminate the simple by the complex when he likens the temperature curve for rate of diastatic action to similar curves for protoplasmic activity. It would be very much more to the point to explain that we know very largely the chemistry and physics of the first curve. Rise of temperature increases the rate of diastatic action with a rather constant coefficient (somewhat less than 2 for each 10° rise). It also increases the rate of coagulation of the enzyme, especially rapidly at higher temperatures, hence the high optimum. The coagulation is a function of the time as well as of the temperature. This accounts for the optimum being much lower when the duration of the experiment is great. The failure of JOST to apply the idea of time as a factor in coagulation of proteins by heat is evident a number of times in the book, especially in his dismissal of LEPESCHKIN'S conclusion that death in general from supramaximal temperatures is due to the coagulation of cell proteins. While at one point JOST accepts and clearly discusses BLACKMAN'S conclusions on "optima and limiting factors," he frequently lapses into the older German view and phraseology. JOST'S arguments in favor of the claim that rate of water absorption by a cell as affected by temperature is a matter of protoplasmic regulation are rather weakened by the recent work of BROWN and WORLEY on barley grains, in which they found that temperature effects appear with about the same coefficient when non-living membranes only or chiefly are involved. Many of these criticisms are an outgrowth of JOST'S peculiar brand of vitalism. If he is convinced beyond a doubt that a physical or chemical explanation holds, he accepts it, but at too many points in his lectures he fails to outline the attack along these lines.

In spite of its virtues in organization, the book could be greatly improved by better organization of the materials within the chapters and by a far more extensive index. At many points the author makes extensive detours from the subject under discussion. Graduate students frequently complain that they must read certain lectures several times and finally reorganize them before they can be held in mind. Paragraph headings such as BARNES or NATHANSOHN have used would serve the double purpose of making the plan of organization more evident and perhaps of leading to a better plan. An author index separate from or as a part of a more extensive general

index would render the material of the book more accessible.—WILLIAM CROCKER.

Biochemistry of plants

In revision, the first volume of CZAPEK'S *Biochemistry of plants*[2] has grown from 584 pages to 828 pages. The historical introduction contains 18 pages as in the previous edition. The portion on "general biochemistry" contains 220 pages as compared with 77 pages in the old edition. Under "special biochemistry" the first part ("The sugars in the metabolism of plants") has been increased from 397 pages to 469; and the second part ("The lipoids in the metabolism of plants") has grown from 94 pages to 112. One of the most notable changes in the general organization of the book is the treatment of the lipoids after the sugars instead of before. This seems desirable because of the order of synthesis of the two groups of substances in the plant.

The "general biochemistry" contains the two chapters of the old edition, "The substratum of the chemical processes in the living organism" and "Chemical reactions in the living plant organism," with two additional chapters entitled "Chemical stimulation effects" and "The chemistry of adaptation and heredity." In this part one is impressed with the excellent summary of the literature on general characters of colloids, gels and adsorption phenomena, catalysis, general chemistry of enzymes, and kinetics of enzyme action.

The part on sugars in the metabolism of plants shows few changes in organization. The additional space used is largely due to the growth of the literature of the subject.

The part on lipoids is divided into two sections, "The nutrient lipoids of the plant" and "The cytolipoids of plants." In the first section, the chapter headings are identical with those of the first edition: "The reserve fats of seeds," "Resorption of fats in seed germination," "Fat synthesis in ripening seeds and fruits," "Reserve fats in stems and leaves," "Reserve fats in thallophytes, mosses, ferns, and pollen grains." The section on cytolipoids has undergone some changes in organization and more in content. The chapter headings are "Plant cerebrosides," "Sterinolipoids of plants (phytosterol and related bodies)," "Plant chromolipoids," and "The production of wax."

The table of contents has been greatly improved by the addition of heads and subheads, giving a much better grouping of the chapters. The treatment of such subjects as photosynthesis, alcoholic fermentation, respiration, and other plant processes reminds one that the work is by no means a plant chemistry in the narrow sense of the word. It is more nearly a physiology of metabolism in plants, with main emphasis on the fundamental chemistry and physics involved in the processes.—WILLIAM CROCKER.

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[2] CZAPEK, FR., Biochemie der Pflanzen. 2d ed. Vol. I. pp. xix+928. Jena: Gustav Fischer. 1913.

Physiological plant anatomy

An English translation of the fourth edition of HABERLANDT'S *Physiologische Pflanzenanatomie*[3] will be welcomed by all who have been engaged in the study and teaching of this particular phase of the science, particularly as it will make the investigations of this leader in physiological anatomy more readily available for students. As pointed out by the translator, the latest German edition, recently reviewed in this journal,[4] may be assumed to embody the mature and considered views of its author with regard to this section of botanical science. In the chapter on sense organs, in particular, there is much original data, now appearing for the first time in English. Here no one will question the facts presented, although many will object to the teleological interpretations given by HABERLANDT.

The translator seems to have done his work very well, at times using considerable freedom to obtain a desirable clearness of expression quite in keeping with the meaning of the original. The volume is well printed in a most legible type, and all the illustrations, notes, and bibliography of the German edition are retained. One might sometimes wish, however, for complete citations of the literature.—GEO. D. FULLER.

MINOR NOTICES

A northwestern manual.—FRYE and RIGG[5] have prepared a manual for the use of schools of Oregon, Idaho, Washington, and the coastal region of southwestern British Columbia. These special manuals for relatively restricted regions are very useful for schools, since the keys can be made much more direct and simple, and the descriptions can be fitted more closely to the local conditions than is possible in manuals that cover a large area. The book is well organized, with every device for easy use, and should prove well adapted to its purpose. The real test of a manual lies in its use, and the reviewer cannot estimate this one from such a standpoint, but he has every reason to believe that the long experience of the authors in the region covered has enabled them to fit the work exactly to its purpose.—J. M. C.

Plantae Wilsonianae.—SARGENT[6] in co-operation with several specialists has recently issued, as a fourth part of *Plantae Wilsonianae*, another important

[3] HABERLANDT, G., Physiological plant anatomy, translated from the 4th German edition by Montagu Drummond. 8vo. xv+777. *figs. 291*. London: Macmillan. 1914. $6.50.

[4] BOT. GAZ. 55:402. 1913.

[5] FRYE, T. C., and RIGG, G. B., Elementary flora of the Northwest. pp. 256. New York, Cincinnati, and Chicago: American Book Co. 1914.

[6] SARGENT, CHARLES SPRAGUE, Plantae Wilsonianae. An enumeration of the woody plants collected in western China for the Arnold Arboretum of Harvard University during the years 1907, 1908, and 1910 by E. H. WILSON. Part IV. Publications of the Arnold Arboretum. no. 4, 8vo. pp. 262. Cambridge: The University Press. Issued March 24, 1914.

contribution to our knowledge of the flora of China. The present part, like the preceding ones, is based primarily on a critical study of plants collected in western China by E. H. WILSON, but it includes also citations of collections made by HENRY, FAURIE, JACK, PURDOM, SARGENT, TAQUET, and others. Upward of 100 species and varieties new to science are recorded in some 40 different genera. The importance of the work lies, not only in the record of new plants, but also in the incorporation of much synonomy and bibliography from scattered publications.—J. M. GREENMAN.

NOTES FOR STUDENTS

Hereditary symbiosis.—One of the most remarkable of recent botanical discoveries, that of hereditary symbiosis between bacteria and seed plants, was made independently by MIEHE and VON FABER,[7] thus adding another to the notable list of great simultaneous achievements. As early as 1894 TRIMEN noted the persistent presence of small knotlike excrescences on the leaves of certain tropical Rubiaceae of Ceylon. In 1902 ZIMMERMANN noted the constant presence of bacteria in these structures, at least in four species from Java, whereupon he referred to them as bacterial knots (*Bacterienknoten*). Since ZIMMERMANN did not take up the question of the origin of the bacterial knots, VON FABER went to Buitenzorg in 1910 to make an extended study of them. A preliminary report of his early observations was made in 1911, and a full account followed in 1912.[8]

VON FABER investigated the symbiotic relations of five species of Rubiaceae, viz., *Pavetta indica*, *P. angustifolia*, *P. lanceolata*, *P. Zimmermanniana*, and *Psychotria bacteriophila*. In the closed buds the bacteria are found in resinous masses in among the leaf primordia. As the leaves develop, the bacteria enter them through certain precociously appearing stomata and pass into intercellular spaces, Soon there is differentiated in the leaf a special tissue composed of small cells rich in chlorophyll. Between these cells there develop capacious intercellular spaces, which the bacteria occupy henceforth. By the time the bacteria have occupied these spaces, the precocious stomata through which they entered the leaf become closed. From pure cultures of the host plants it was discovered that the bacterial tissue is derived from primordia which without the presence of the bacteria develop into a secretory reservoir, in which there accumulates a resin similar to that noted above as present in the bud. It is probable that the bacteria are attracted by this resin. Careful study of every stage in the life history of the host plants showed that the bacteria are always present. They become inclosed in the ovary at flowering,

[7] VON FABER, F. C., Über das ständige Vorkommen von Bakterien in den Blättern verschiedener Rubiaceen. Bull. Dép. Agric. Ind. Néerl. **46**: pp. 3. 1911. (See Bot. Centralbl. **119**:351. 1912.)

[8] ———, Das erbliche Zusammenleben von Bakterien und tropischen Pflanzen. Jahrb. Wiss. Bot. **51**:285–375. *figs. 7. pls. 3.* 1912.

and they enter the embryo sac through the micropyle. In seeds they occur constantly between the embryo and the endosperm. They are seen in young seedlings and in all later stages.

Careful study was made of the bacteria, both in the host plants and in artificial cultures. They were found to bear a close resemblance to the tubercle bacillus, both in structure and in behavior. Consequently VON FABER regards these organisms as members of the Mycobacteria, and he gives them the name *Mycobacterium Rubiacearum*, nov. sp. Of large interest also was the discovery that nitrogen is fixed in the artificial cultures of these organisms. VON FABER succeeded also in getting pure cultures of the Rubiaceae, the seeds being sterilized by placing them for 25 minutes in hot water at a temperature of 50°. The pure cultures grew far less vigorously than symbiotic cultures, and the leaves were lighter in color. It was observed also that nitrogen is fixed in the symbiotic cultures but not in the pure cultures, so that in nature these Rubiaceae can get their nitrogen supply directly from the air. Still further recalling the nutritive relations between the Leguminosae and their root bacteria is the fact that the bacterial tissue of the leaves of the Rubiaceae is rich in starch, which may serve the bacteria for food; there is evidence also of bacterial decadence, involution forms, and eventual phagocytosis. Pure cultures seem to indicate that each host species has its own bacterial "adaptation form."

MIEHE has also followed brief preliminary reports[9, 10] of his studies of hereditary symbiosis by detailed accounts.[11, 12] *Ardisia crispa*, one of the Javanese Myrsinaceae, has glandular thickenings on the leaf margins. These represent modified hydathodes and have commonly been regarded as albumen glands. It is now shown that these structures resemble ZIMMERMANN'S bacterial knots and are caused by bacteria. Furthermore the bacteria are present throughout the life history of *Ardisia*, and the details of bacterial entrance and subsequent behavior are astonishingly like those reported simultaneously in the Rubiaceae by VON FABER. The microorganisms enter the leaves through stomata, which later become closed. They were observed by MIEHE in the embryo sac, in the seed, and in the vegetation point of the seedling. Two species of bacteria have been isolated and are called *Bacillus foliicola* and *B. repens*. In old cultures there occur curved and branched involution forms. In most respects these organisms resemble VON FABER'S

[9] MIEHE, H., Die sogenannten Eiweissdrüsen an den Blättern von *Ardisia crispa* A.DC. Ber. Deutsch. Bot. Gesells. 29:156–157. 1911.

[10] ———, Über Symbiose von Bakterien mit Pflanzen. Biol. Centralbl. 32:46–50. 1912.

[11] ———, Die Bacterienknoten an den Blatträndern der *Ardisia crispa*. In Javanische Studien. Abhandl. Königl. Sächs. Gesells. Wiss. 32:398–431. 1911.

[12] ———, Weitere Untersuchungen über die Bakteriensymbiose bei *Ardisia crispa* I. Die Mikroorganismen. Jahrb. Wiss. Bot. 53:1–54. *pls. 2*. 1913.

Mycobacterium, except that there is no evidence of the fixation of nitrogen. Hitherto pure cultures of *Ardisia* have been unobtainable.

VON FABER[13] very recently has published further, checking up various minor points, and discussing MIEHE's work and his criticisms of the work of VON FABER. The most noteworthy result recorded in the latest paper is the success of the attempt to synthesize pure cultures of *Pavetta* and *Mycobacterium*. A symbiosis of the usual kind seen in nature resulted from the inoculation of the former by the latter. The luxuriant cultures thus arising seem to show clearly that VON FABER was working with the proper symbionts.—H. C. COWLES.

Stomatal activity.—ILJIN[14] has found that when the stomates of *Centaurea orientalis* are open, the guard cells have an osmotic pressure ranging from 85 to 108 atmospheres. When the stomates are closed, the guard cells have an osmotic pressure of 13–20 atmospheres. The osmotic pressure of the epidermal and parenchyma cells of the leaves vary little and approximate that of the guard cells with the stomates closed. Similar results were obtained for *Senecio Doria, Iris pumila, Eryngium campestre, Verbascum Lychnitis, Veronica incana*, and others. The guard cells with high osmotic pressure (stomates open) contain no starch, while the guard cells of low osmotic pressure (stomates closed) bear an abundance of starch. Conditions that bring about the closure of the stomates, darkness or excessive transpiration, will produce the condensation of the sugar to starch, accompanied by the great fall in osmotic pressure in about two hours. The reverse process of hydrolysis, accompanied by the great rise of osmotic pressure and opening of the stomates, is accomplished in about the same time under illumination and low evaporation power of the air. If these results are correct, we have here a great contribution to the mechanics of stomatal regulation. One would like to know the variation in the osmotic pressure of guard cells that show little stomatal regulation, as is true of certain swamp and xerophytic forms.

ILJIN[15] has also made an extensive study on stomatal regulation of transpiration. He used cuttings of plants in potometers and calculated the transpiration on the basis of the grams loss of water per 1000 cm.^{2} per hour. While the potometer measures water absorption rather than loss, he believes that the two quantities are essentially equal in his work, since he has always discarded experiments in which wilting became noticeable. He ran his experiments in the open, either in an exposed place (the steppe) or in a protected region

[13] VON FABER, F. C., Die Bakteriensymbiose der Rubiaceen (Erwiderung und ergänzende Mitteilungen). Jahrb. Wiss. Bot. **54**:243–264. *figs. 3.* 1914.

[14] ILJIN, W. S., Die Regulierung der Spaltoffnung im Zusammenhang mit der Veränderung des osmotischen Druckes. Beih. Bot. Centralbl. **32**:15–35. 1914.

[15] ———, Die Probleme des vergleichenden Studiums der Pflanzentranspiration. Beih. Bot. Centralbl. **32**:36–65. 1914.

(the ravine). On the steppe, *Sanguisorba officinalis* transpired more rapidly than *Clematis integrifolia*, 3.3 gm. against 1.2 gm. In the ravine the reverse was true, 1.7 gm. against 0.7 gm. Similar results were found for *Phlomis pungens* and *Ajuga Laxmanni*, with the former transpiring more rapidly in the steppe and less rapidly in the ravine. These facts are explained by the rapid closure of the stomates of *Sanguisorba* and *Ajuga* in the exposed position, and the slow closure in the other forms. In another experiment, two marked xerophytes, *Aster villosa* and *Veronica incana*, showed much higher transpiration than two evident mesophytes, *Aristolochia clematitis* and *Sanguisorba officinalis*. The losses in these forms were respectively 43.3, 15, 11.3, and 6.4 gm. These results are explained by the stomates being closed in the mesophytes and open in the xerophytes.

It was rather a common thing to find higher transpiration in a given mesophyte in the ravine than on the steppe, owing to the stomatal closure in the latter place. In *Ajuga Laxmanni*, there were 10.5 gm. on the steppe against 26.2 gm. in the ravine; in *Centaurea orientalis*, 17.6 gm. against 31.5 gm. In an experiment with *Helianthus annuus*, *Pisum sativum*, *Vicia Faba*, and *Polygonum fagopyrum* placed in a series of positions where the evaporation power of the air graded from a low value to a high, the transpiration rose with the evaporation power of the air up to a certain height, then it fell enormously with the rise of the evaporation power of the air. The break was the point at which stomatal closure was induced. When the author started with various mesophytes and xerophytes, all with the stomates open, and placed them in conditions where the evaporation power of the air was rather high and rising rapidly, as time elapsed the mesophytes showed rapid transpiration, rising rapidly with the evaporation power of the air for two hours or so, followed by a rapid fall. In this case there is a high and sharp pointed curve. The xerophytes showed slower initial transpiration, a far slighter rise with the evaporation power of the air, and no such marked fall. In this case, the curve is flat, with no very high or sharp point. ILJIN believes the two types of curves represent mesophytism on the one hand and adaptation for xerophytism on the other. The xerophyte can protect itself against excessive transpiration in exposed positions without curtailing extremely the CO_2 necessary for carbon assimilation by rapid or extreme stomatal closure. This work indicates the great importance of stomatal variation in regulating transpiration, especially in mesophytes, a conclusion quite in contrast with that of LLOYD.—WILLIAM CROCKER.

Alpine plant-geography.—RYDBERG,[16] in the first three of a series of articles on the phytogeography of the Rocky Mountain region, has discussed the alpine zone, its environmental conditions, geographic floristics, and plant

[16] RYDBERG, P. A., Phytogeographical notes on the Rocky Mountain region. I. Alpine region; II. Origin of the alpine flora; III. Formations in the alpine zone. Bull. Torr. Bot. Club 40:677–686. 1913; 41:89–103, 459–474. 1914.

communities. The first article characterizes the alpine zone as the area below perpetual snow and above the forest line, including for convenience the transitional area in which subalpine *Krummholz* and scattered trees alternate with alpine grassland. A number of the factors which together determine the position of timber line are discussed. The writer's opinion that deficiency of rainfall, or of precipitation in proper form, is an important factor preventing tree growth in the alpine zone may be questioned. In the table of precipitation for various points in Colorado given by ROBBINS (BOT. GAZ. 49:260. 1910), Pike's Peak has the highest mean annual rainfall listed, 28.65 inches; July and August, when alpine plants are most active, are the rainiest months. The influence of mountain masses is given by RYDBERG as tending to moderate conditions, so as to allow trees to extend to higher elevations. This effect may not be so general, for some botanists believe the presence of surrounding mountain masses have the opposite influence in some cases. Few observers would agree with RYDBERG in classing Long's Peak, in the Front Range, with Pike's Peak and Sierra Blanca as isolated mountains. The question may be regarded as still open.

In the second article the regions in which the alpine species have probably originated (perhaps it would be better to say the geographic sources from which they have probably been derived) are discussed, with lists for each region and lists of species common to two or more regions. The author is of the generally prevalent opinion that most of our alpine plants reached their present scattered stations during glacial times, when the circumpolar arctic-alpine flora was practically continuous over much lower latitudes and altitudes than at present. More than one-third of the species are restricted to North America. About 100 species from the subalpine zone extend higher than the forest line, and the purely alpine plants number about 250 species.

The third article is an account of the associations. The communities are called formations (in the specific sense in which the term association is generally used); they are based on habitat as determined by topography. They are: (1) rock-slide formation of rock-slides and rock-fields; (2) the generally distributed mountain crest formation of areas thinly covered with gravelly soil (apparently this corresponds to the dry meadow of COOPER, BOT. GAZ. 45: 324. 1908); (3) mountain seep formation; (4) alpine meadow; (5) alpine bog; (6) alpine lake or pond; (7) cliff formation; (8) snowdrift formation. It is perhaps a question whether some of the associations might not better have been characterized and named from the vegetation itself rather than from the habitat. The lists of species are full; those extending into the lower mountains are distinguished, and also those characteristic in, or confined to, the northern or southern parts of the Rocky Mountain region. Hardly any information is given to indicate which species are most frequent, abundant, or characteristic in particular associations. The alpine zone is really well known to very few botanists, and articles dealing with its vegetation are not numerous, thus giving the present writing so much the greater value.—ARTHUR G. VESTAL.

The mycorhiza of forest trees.—Some important experiments have been carried on by JOSEF FUCHS[17] with the mycorhiza of forest trees. The chief object of the experiments was the synthetic production of mycorhizas by bringing together pure cultures of the two symbionts. The trees employed were various conifers, and the fungi consisted of a number of humus-inhabiting forms. Most of the experiments gave negative results, but when six-month seedlings of *Pinus Strobus* were brought into contact with cultures of *Collybia macroura*, a strong development of endotrophic mycorhiza was secured. The finding in certain cultures of spores and mycelia quite unlike those used in the inoculations caused FUCHS to believe that root infection often may come from the seeds rather than from the substratum. *Picea* seedlings eight days old growing in sterilized humus had their roots infected by fungi. The infected cells of the conifer roots soon turned brown and were cast off, suggesting that the fungi are truly parasitic and not beneficial to the conifers. Frequently the invading fungi are deformed and killed by the protoplasm of the root. These results were obtained both with ectotrophic and with endotrophic mycorhizas.

W. B. McDOUGALL[18] has made a careful study of the mycorhizas of a number of our common American trees, chiefly angiospermous species. Various forms of ectotrophic and endotrophic mycorhizas are described, both sorts being found on the same root in *Tilia americana*. In some cases the fungus species involved were identified, and it was observed that the mycelia of different species frequently can be distinguished from one another by differences in color and structure. Observations made at all seasons showed that mycorhizas are much more in evidence in autumn, winter, and spring than in summer, and hence are usually annual. The fungal symbiont in ectotrophic mycorhizas, so far as known, is almost always a basidiomycete, whereas this is rarely the case with endotrophic mycorhizas. Some mycorhiza fungi can inhabit several hosts and the host trees also may have several different mycorhiza fungi, but all mycorhizal fungi cannot form mycorhizas on all mycorhizal trees. Individual trees or parts of trees are often without root fungi, probably because the proper fungus species happens to be absent. The development of the mycelial mantle in the ectotrophic mycorhizas checks further root growth, whereupon branching takes place, resulting in the characteristic coralloid aspect of the small root branches. The author in discussing the theories of previous workers agrees with FUCHS (though not quoting him) that the fungi of ectrotrophic mycorhizas are ordinary parasites; these fungi are of no value to the trees, nor are they probably very harmful, since so many roots are without them, especially in the deeper soil layers. McDOUGALL is less confident concerning the

[17] FUCHS, JOSEF, Über die Beziehungen von Agaricineen und anderen humusbewohnenden Pilzen zur Mycorhizenbildung der Waldbäume. Bibl. Bot. no. 76. pp. 32. *pls. 4.* 1911.

[18] McDOUGALL, W. B., On the mycorhizas of forest trees. Amer. Jour. Bot. 1:51–74. *pls. 4. fig. 1.* 1914.

rôle of the fungi in endotrophic mycorhizas, as in *Acer*. The only adverse criticism to be made of this excellent paper concerns the mere detail of the use of the words symbiosis and heterotrophic. Heterotrophic is used for the case (*Tilia*) where the same root has ectotrophic and endotrophic mycorhizas, which certainly is not the usual sense of the word. Both McDougall and Fuchs contrast parasitism and symbiosis, whereas etymology and the best usage make parasitism a kind of symbiosis.—H. C. Cowles.

Photo-growth reaction.—Blaauw,[19] who has already proved himself a master in phototropism, now publishes an excellent piece of work on the effect of illumination on the growth of the sporangiophore of *Phycomyces*. He uses the term "photo-growth reaction" to indicate the changes in growth rate and amount caused by a single short application of light. He first works with equilateral illumination applied at right angles to the organ from four or eight directions. The quantity of illumination in the different experiments varies from 1 to 7,680,000 M.K.S. In all cases an early acceleration in growth is followed by a later retardation. In illumination of 16 M.K.S. and above the acceleration begins about 3.5 minutes after the beginning of illumination. In 1 M.K.S. it begins after 8 minutes, and in 6 M.K.S. after 6 minutes. The maximum acceleration was at about 7 minutes in 16 M.K.S. and above and later in lower quantities. Then follows a gradual fall in growth rate until a rate considerably below the normal is reached, and then a gradual rise until the normal rate is again reached. The duration, amount, and overlapping of these reactions vary much with the amount of illumination. In some of the lower light amounts the total acceleration exceeds the total retardation by threefold, while in the higher amounts the latter exceeds the former. This agrees with the finding of Jacobi that slight illumination (low intensities of medium duration or high intensities of short duration) accelerate growth, while medium or great amounts of illumination retard growth. Jacobi deals with only the difference of the accelerating and retarding effects, since she took her readings 24 hours after exposure. Blaauw's work gives the continuous curves. In all the older works only the retarding effect had been reported. Blaauw finds that for low light quantities, where the accelerating does not overlap the retarding effect, a quantitative relation can be found between quantity of stimulus and quantity of acceleration. The increased growth is proportional to the cube root of the light amount. Jacobi's conclusion that the quantity of stimulus law does not apply here is due to her failure to recognize that both effects (accelerating and retarding) appeared in every application, and that she was dealing only with their difference.

In a second group of experiments Blaauw deals with phototropic response in the same organ, and with good evidence comes to the conclusion that phototropism in this form can be explained entirely by the total of the "photo-growth reactions." This brings us back to the old view of De Candolle under

[19] Blaauw, A. H., Licht und Wachstum I. Zeitsch. Bot. 6:641–703. 1914.

a more complex garb. BLAAUW's work clearly indicates that the amount of effective light and not the direction of the ray is the determining factor in phototropism. He believes NOAK's[20] opposing view is due to his overlooking the parabolic curve of the tip in the epicotyl of *Avena*, the shading effect of the sporangia of *Phycomyces* on the perceptive and growth regions of the sporangiophore, and the cylindrical lens effect of the latter organ. On account of its lens action the back of the organ in unilateral light is more strongly illuminated than the front. The matter is rendered more complex by the focal line lying at different depths with variation in the angle of the incident ray.—WILLIAM CROCKER.

The vegetation of Natal.—Perhaps no part of the world is theoretically more interesting and practically less known to the phytogeographer than South Africa, and it is a satisfaction to record the appearance of two excellent papers on the vegetation of Natal by Professor BEWS[21,22] of the Natal University College. The first paper is of general nature, presenting the ecological factors and plant associations of the province as a whole. Although Natal is situated considerably to the south of the Tropic of Capricorn, much of the area is frostless and has a distinctly tropical vegetation. Especially is this true of the coast, where are to be found such tropical types as the mangroves and Pes-caprae. Almost all of the coast line is fringed by dunes, reaching a height of 50–200 feet, and covered chiefly by xerophytic bush. The vegetation of the interior is mostly evergreen dicotylous forest and grassland. The forest (generally called bush) resembles SCHIMPER's sclerophyll forests, except that they are in regions of summer rather than winter rain. Perhaps the most interesting type of bush is the yellow-wood bush, in which *Podocarpus* dominates. In the Natal bush epiphytes are relatively scarce, but lianas are very abundant. Transitional to the grassland or veld is the thorn veld, essentially a savanna, with a dominance of umbrella-shaped *Acacia* trees. In the veld the grasses are changing, largely because of human influences, and it is noteworthy that the invading grasses are less useful to man than the original grasses. A brief account is given of the marsh or vlei and of secondary associations, that is, those due to human influence.

The second paper is the initial one of a series contemplated by BEWS, dealing in detail with the vegetation of small areas in the province of Natal. In the veld the dominating natural grass is *Anthistiria imberbis;* increasing areas are being given over to the cultivation of wattle (*Acacia mollissima*)

[20] BOT. GAZ. 58:88–89. 1914.

[21] BEWS, J. W., The vegetation of Natal. Annals of the Natal Museum 2^3:253–331. *pls. 10.* 1912.

[22] ———, An ecological survey of the midlands of Natal, with special reference to the Pietermaritzburg district. Annals of the Natal Museum 2^4:485–545. *pls. 7. map 1.* 1913.

and maize (known locally as mealie), and vast areas have been extensively modified by grazing and burning. In the modified veld *Aristida junciformis* largely replaces *Anthistiria*. The bush, vlei, and other types of associations are much less extensive about Pietermaritzburg than are those of the veld. The paper is accompanied by a map, indicating the areas occupied by the different associations.—H. C. COWLES.

The origin of coal.—A recent bulletin[23] from the Bureau of Mines presents the results of extensive researches as to the origin of coal, long a vexed question. WHITE discusses the geologic relations of coals, analyses of coal samples studied under the microscope, physiographic conditions attending the formation of coal, rate of deposition of coal, and regional metamorphism of coal. DAVIS contributes an account of the origin and formation of peat; while THIESSEN describes in detail the results of a microscopic study of coal, prefacing his account with a full historical review of the subject. The bulletin is so full of important facts and interesting inferences that it is impossible to recount them here, but some of the general conclusions may be mentioned.

An important conclusion is that all coal was laid down in beds analogous to the peat beds of today; and that all kinds of plants, in whole or in part, went into the deposit. The various materials entering into the structure of plants differ widely in their resistance to the various agencies that were concerned in peat formation and in the subsequent coal formation. At the death of the plants, dependent upon the conditions in the bog, a partial decomposition, maceration, elimination, and chemical reduction begins, brought about chiefly by organic agencies, mainly fungi at first, and later bacteria. Such labile substances as proteins are removed first, and the more resistant next, leaving the most resistant in the residue called peat. The various processes referred to above, conducted chiefly by biochemical agencies, are taken up and continued by "dynamochemical" agencies, through various later stages, resulting in the different grades of coal, as lignite, subbituminous, bituminous, cannel coal, and anthracite. "Coal, therefore, is chiefly composed of residue consisting of the most resistant components, of which resins, resin waxes, waxes, and higher fats, or the derivatives of the compounds composing these, are the most important." These substances perform mainly protective functions in plants, as in cuticles, spore exines (including pollen), bark, cork, and waxy coverings. A very interesting result of these investigations is that any algal origin of coal was not demonstrated, although this has been a conspicuous and perhaps favorite theory.—J. M. C.

Water requirement of plants.—The ratio of the amount of water taken up by a plant during its growth to the dry matter produced has been found to vary very much, and it would seem that its careful investigation would

[23] WHITE, DAVID, and THIESSEN, REINHARDT, The origin of coal. Bull. 38, Dept. Interior, Bureau of Mines. pp. x+390. *pls. 54.* 1913.

throw light upon the questions of what crop plants make most economical use of water and of what wild plants are best suited to their desert and semi-desert habitats. A former review[24] has called attention to the investigation of these problems by SHANTZ and BRIGGS during 1910 and 1911, while a more recent paper reports the results of the same investigators[25] obtained during the summers of 1912 and 1913. The investigations are remarkable for the extensive scale upon which they have been conducted, and for their duration throughout the growing season. More than 50 species have been the subjects of study, and for some the period of investigation extends over three years and includes many individual plants grown from seedling to maturity, the final result being the average of many determinations. As a rule, the same variety gave consistent results, although considerable differences were found between different varieties of the same plant; for example, the variety of alfalfa having the highest water requirement was nearly 50 per cent above the lowest.

Millet has proved throughout an excellent dry land crop, producing a unit of dry weight for every 310 units of water absorbed. It is closely followed by sorghum with a water requirement of 322, corn with 368, and sugar beet with 397; then come wheat with 513, barley with 534, oats with 597, alfalfa with 831, and others that it is impossible to enumerate here. Weeds show the greatest known range from such economic forms, as *Amaranthus* with 292, *Salsola pestifer* with 336, *Bouteloua gracilis* with 389, through such intermediate forms as *Xanthium commune* with 432, *Grindelia squarrosa* with 608, and *Helianthus petiolaris* with 683, up to *Ambrosia artemisiaefolia* with 948 and *Agropyron Smithii* with 1076. Like previous investigations by the same workers, this report contains a vast amount of exact quantitative data of value in studying the agricultural possibilities and the ecology of the great plains.—GEO. D. FULLER.

The origin and relationships of the Indonesian flora.—It is well known that WALLACE, basing his conclusions chiefly on animals, held to the idea of a sharp boundary line in the Straits of Macassar, separating the Indo-Malay and Australasian biogeographic regions. Not only were Borneo and Celebes thus separated biogeographically, but the line was supposed to separate such closely adjoining islands as Bali and Lombok, east of Java. Botanists generally have not found sharp lines between the Malay and Australian floras. HALLIER,[26] working under excellent auspices, finds that Asiatic types extend

[24] BOT. GAZ. 56:514–515. 1913.

[25] BRIGGS, L. J., and SHANTZ, H. L., Relative water requirement of plants. Jour. Agric. Research 3:1–63. *pls. 7*. 1914.

[26] HALLIER, HANS, Die Zusammensetzung und Herkunft der Pflanzendecke Indonesiens. Separate reprint from J. ELBERT's Die Sunda-Expedition des Vereins für Geographie und Statistik zu Frankfurt am Main 2:275–302. *figs. 2*. 1912.

far into Polynesia, fading out gradually instead of stopping abruptly. Similarly the Polynesian types extend into Indonesia, ceasing gradually and not suddenly. Thus phytogeographers are given more solid reasons than ever for opposing the view of WALLACE. Starting from this sure foundation, HALLIER sets out on the perilous task of constructing land bridges between present-day islands and continents. He believes that Indonesia, Australia, and Polynesia were once connected, the islands now existing having been the mountain peaks of this former continent. In still older times HALLIER believes that Australasia and Polynesia were connected by a wide land bridge with America, the northern boundary extending through the Sandwich Islands to Lower California and the southern boundary extending from the southern islands of New Zealand, south of the Society Islands, through Easter Island and Juan Fernandez to southern Chile. HALLIER's views recall the submerged continent postulated by DARWIN in connection with his theory of the origin of coral islands; nowadays, however, geologists seem to be getting more and more convinced of the relative permanency of oceans and continents, at least throughout the more recent ages. The possibilities of plant migration in our present world are so very large that botanists may well leave to the zoologists the construction of extensive land bridges and the arbitrary submergence and emergence of continents.—H. C. COWLES.

Evaporation and plant succession.—Among the recent contributions of quantitative data concerning the factors causing the succession of plant associations is a study by WEAVER[27] of the evaporation conditions within certain grassland and forest associations of Washington and Idaho. The succession is from the prairie to a climax forest of cedar (*Thuja plicata*), and the record extends over 126 days beginning May 7, 1912. The average daily amounts of evaporation for the various associations taken in the order of their occurrence in the succession are, approximately, bunch grass 28 cc., prairie with southwest exposures 23 cc., prairie with northeast exposure 17 cc., yellow pine (*Pinus ponderosa*) 12 cc., fir-tamarack 9 cc., and cedar forest 8 cc. These atmospheric conditions are further compared, and using those of the mesophytic cedar forest as the standard of reference, it is found that "in the fir-tamarack association from May to September, atmospheric conditions in the lower stratum are 120 per cent as severe, in the average prairie of the plains 250 per cent, and in the bunch grass association 345 per cent as unfavorable for plant life as regards the evaporating power of the air." Moreover, the conditions in the mesophytic forest are found to be almost identical to those recorded by the reviewer[28] for the climax mesophytic forest of the eastern United States as determined in

[27] WEAVER, J. E., Evaporation and plant succession in southeastern Washington and adjacent Idaho. Plant World 17:273–294. 1914.

[28] FULLER, G. D., Evaporation and plant succession. BOT. GAZ. 52:193–208. 1911.

beech-maple forests of the Chicago region. Thus it is possible to make rather accurate comparisons of the conditions within the forests of the east and the west and to obtain quantitative demonstration of the equal mesophytism of the latter.

The differences in the evaporating power of the air in the different associations are found to be quite sufficient to show that this factor must be an important one in causing succession. Such accumulations of quantitative data as are contained in the present paper mark the advance of ecology along lines tending toward greater exactness, and it is to be hoped that they will become increasingly numerous.—GEO. D. FULLER.

Phylogeny of Filicales.—In continuing his studies of the Filicales, BOWER[29] has investigated *Blechnum* and its allies, and finds that the characters of the sori are of most importance in suggesting phylogenetic lines. The genus is treated in its wider sense, as comprising the subgenera *Lomaria*, *Salpichlaena*, and *Eu-Blechnum*. In *Lomaria* the indusium appears marginal, while in *Eu-Blechnum* it becomes apparently intramarginal owing to the formation of a new structure which BOWER calls the "flange." He produces evidence from a comparison of the development in numerous species that the protective organ is phyletically the same throughout the genus *Blechnum*, and he calls it the "phyletic margin." The general conclusions reached are as follows.

The *Blechnum*-like ferns and their derivatives represent a true phyletic sequence, which is traced to the region of the Cyatheaceae, the actual point of contact probably being *Matteuccia intermedia*, a fern of North China recently described by CHRISTENSEN. From this source several divergent lines have proceeded, the main line leading through § *Lomaria* to *Eu-Blechnum*, involving the origin of the "flange" and the diversion of the "phyletic margin" to indusial functions. Minor lines led to *Acrostichum*-like derivatives in *Stenochlaena* and *Brainea*. Interruption of the fusion sorus, occurring as an anomaly in *Blechnum*, led to the conditions shown in *Woodwardia* and *Doodia*. An outward arching of the fusion sorus of *Blechnum*, ultimately combined with interruption, gives the key to the origin of *Scolopendrium*. An outward swinging of the interrupted fusion sori, variously combined with archings and new formations of partial sori, and various branchings of the leaf, give the several types of *Asplenium*. The relation of *Plagiogyria* to the whole series is regarded as problematical, but it is suggested that it is an isolated and relatively primitive genus.—J. M. C.

Evolution of inflorescence.—PARKIN[30] has studied inflorescence from the evolutionary point of view, a subject which in his judgment has been "strangely

[29] BOWER, F. O., Studies in the phylogeny of the Filicales. IV. *Blechnum* and allied genera. Ann. Botany 28: 363–431. *pls. 22–32. figs. 26.* 1914.

[30] PARKIN, J., The evolution of the inflorescence. Jour. Linn. Soc. Bot. 42: 511–563. 1914.

neglected." Textbooks define inflorescences, but there has been no serious attempt to relate them from the standpoint of their evolution. Some of the conclusions from his comparative studies are as follows: flowers were originally borne on the plant singly, each terminal to a leafy shoot; from such a shoot, bearing foliage leaves below and ending in a single terminal flower, all inflorescences, as well as the solitary axillary flower, have probably arisen; two main classes of flower clusters are distinguished, which are named "apical" and "intercalary," the majority of inflorescences belonging to the former class, which includes the long recognized cymose and racemose types. The author carries these preliminary propositions forward into details as to how the various clusters have arisen. For example, the first flower cluster to arise from the solitary terminal flower is said to have been cymose in character. From this start various tendencies are traced, and among the results it follows that racemose inflorescences have proceeded from cymose ones, the panicle being the intermediate stage. In regard to the origin of solitary axillary flowers, the author proposes at least three different ways, all capable of being traced back to the solitary terminal flower. Throughout the presentation the genera showing the various stages in this evolution are cited.—J. M. C.

Ant plants.—ESCHERICH[31] adds further evidence against the now generally discredited theory of myrmecophily, through a study of *Humboldtia laurifolia*, one of SCHIMPER'S typical myrmecophilous plants. Not only do the ants of *Humboldtia* offer it no protection, but they actually bring it harm by attracting woodpeckers. ESCHERICH notes that ants collect and store the bulbs of *Cyperus bulbosus*, and thus may be of significance in the dispersal of the species.

MIEHE[32] has carried on some interesting investigations on *Myrmecodia tuberosa*, one of the most famous of all "myrmecophilous" plants. It appears that the internal walls of the hollow tuber of this plant are in part smooth and yellow and in part warty and black. In the black warty areas the ants (*Iridomyrmex Myrmecodiae*) deposit their excrements, whereas they deposit their eggs in the smooth areas. The black patches owe their color to luxuriant growths of fungi, which doubtless get nourishment from the ant excrements. Possibly the ants use the fungi (which may be *Cladosporium* or *Cladotrichum*) as a source of food, since tufts of mycelia were frequently seen to be shaved off. The warty tracts develop independently of either ants or fungi and are pretty clearly shown to be organs of water absorption. MIEHE believes that the organization of these tubers was related originally to water absorption and accumulation, the ant relation being secondary and incidental.—H. C. COWLES.

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[31] ESCHERICH, K., Zwei Beiträge zum Kapitel "Ameisen und Pflanzen." Biol. Centralbl. 31:44–51. *figs. 2.* 1911.

[32] MIEHE, H., Untersuchungen über die javanische *Myrmecodia*. In Javanische Studien. Abhandl. Königl. Sächs. Gesells. Wiss. 32:312–361. 1911.

———, Über die javanische *Myrmecodia* und die Beziehung zu ihren Ameisen. Biol. Centralbl. 31:733–738. 1911.

Anatomy of the node.—An example of what sort of contribution may be made by real "comparative" anatomy to taxonomy is seen in SINNOTT'S work on the node of Dicotyledons.[33] It has frequently been proposed to use the structure of the petiole in establishing relationships, but this region is subject to too great ecological variation to yield results of general significance. In the basal region of the leaf, however, a simpler and more constant condition is found, and the number of leaf traces is characteristic of great groups. With respect to the Angiosperms it is concluded that the primitive number of traces is three, and that evolution has taken place in two directions: (1) by increase, as in Umbelliflorae; and (2) by reduction to one trace, which appears to happen either by fusion of the original three or by disappearance of the two lateral strands, as may be seen in Cruciferae and Aquifoliaceae respectively. The correctness of these conclusions is attested by the occurrence of transitional forms and by the fact that seedlings frequently show a simpler condition of the leaf trace than does the adult. Such a study supports the validity of a number of ENGLER'S orders, while it casts doubt on certain orthodox views, such as the near relationships of Compositae and Campanulaceae.—M. A. CHRYSLER.

Studies of desert vegetation.—SHREVE[34] has studied the influence of low temperatures on the distribution of the giant cactus, *Cereus giganteus*, and he concludes that the limiting factor in regard to distribution northward is the number of consecutive hours of freezing. Plants exposed experimentally to freezing for six to fifteen hours were not seriously injured, whereas an exposure of more than thirty hours to freezing temperatures resulted in death. It is concluded that the giant cactus cannot exist where an entire day occurs without thawing temperatures. Probably the distribution of many other plants of the warmer deserts are thus limited.

SHREVE[35] has studied also the establishment behavior of the palo verde, *Parkinsonia microphylla*. Out of 542 seedlings of the year 1910, observed in their natural habitats, only 62 remained alive at the end of sixteen months. Further observations showed that a number of seedlings die in the second and third years, whereas most plants attaining the age of three years are fairly established and live for a long time. Physical conditions, rather than competition with other plants, are the chief factor in producing these results, and the most important physical condition is the absorption-transpiration balance.—H. C. COWLES.

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[33] SINNOTT, E. W., Investigations on the phylogeny of the Angiosperms. I. The anatomy of the node as an aid in the classification of Angiosperms. Amer. Jour. Bot. 1:303–322. *pls. 30–34.* 1914.

[34] SHREVE, FORREST, The influence of low temperatures on the distribution of the giant cactus. Plant World 14:136–146. *figs. 3.* 1911.

[35] ———, Establishment behavior of the palo verde. Plant World 14:289–296. 1911.

The plant life of Hartsville, South Carolina.—A delightful account of the flora of his old home town has been given us by COKER.[36] After a brief consideration of the climate, topography, and geology, the various plant formations are considered in turn as follows: sand hills, upland forests, flatwoods, savannas, bays and swamps, lakes and ponds. In the sand hills *Pinus palustris* still dominates; elsewhere its place has been taken largely by *Pinus Taeda*. The resistance of the former to fire is strikingly brought out. *Quercus Catesbaei* is one of the chief forms in the undergrowth. In the upland forests *Quercus falcata* and *Q. velutina* are dominant, although *Pinus palustris* once held an equal place with them. The flatwoods are poorly drained and also are dominated by pines and oaks. The savannas are essentially confined to undrained depressions in the flatwoods. The term bay, a folk-name of the coastal plain, is applied to shallow swamps and is contrasted with the deep swamps. More than half of the work is made up of an annotated list of the Hartsville plants, the trees being annotated more fully than the rest. The chatty, personal touch of this treatise recalls the charming volumes of the older naturalists, who followed in the train of GILBERT WHITE.—H. C. COWLES.

Evolutionary observations from New Zealand.—During the many years which COCKAYNE has devoted to ecological studies in New Zealand, he has, of course, observed many phenomena interesting from the point of view of evolution. These observations are now gathered together into compact form.[37] It is freely admitted that only through careful experiment can exact results be reached, though it is asserted that much valuable experimental material can best be disclosed by ecological study. COCKAYNE cites probable examples of elementary species, variation, and mutation. Epharmony is considered in detail, since it is felt that here is where ecology presents its most important contribution; convergent epharmony, as illustrated by divaricate shrubs, cushion plants, etc., in different families, is especially in evidence in New Zealand. Persistent juvenile forms have often been noted by COCKAYNE, and they are here set forth in some detail. In discussing the struggle for existence, it is noted that the 550 introduced species of New Zealand are by no means submerging the native flora, except in grazed or burned areas. The virgin timber is wholly free from these introduced elements.—H. C. COWLES.

Root characters, ground water, and plant distribution.—CANNON[38] has done much to orient our minds properly with relation to the characters and significance of roots. His discovery of the superficial root systems of cacti

[36] COKER, W. C., The plant life of Hartsville, S.C. pp. 129. *pls. 15*. Published by the Pee Dee Historical Association. Columbia, S.C. 1912.

[37] COCKAYNE, L., Observations concerning evolution, derived from ecological studies in New Zealand. Trans. N.Z. Institute **44**:1–50. *pls. 8. figs. 3*. 1912.

[38] CANNON, W. A., Some relations between root characters, ground water, and species distribution. Science N.S. **37**:420–423. 1913.

has altered our notions concerning the prevailing deep-rootedness of desert plants. In a recent short paper he brings further data along similar lines. The mesquite, as is well known, either may be a shrub or it may be a tree of considerable size. On flood plains, where it is a tree, its roots penetrate to the water table, whose depth may be 15–25 feet. Shrubby specimens on higher grounds have extensively spreading rather than deep roots. CANNON considers the root situation in different types of climate and makes several interesting suggestions.

In a brief note[39] CANNON calls attention to the somewhat curious fact that at Carmel, California, the removal of the chaparral undergrowth in forests of *Pinus radiata* is followed by the death of the pines. This is attributed to the shallow root system of the pines, which comes to grief when the soil is desiccated as a result of the removal of the chaparral.—H. C. COWLES.

Sand hill forestation.—Some government experiments of considerable interest to ecologists are being conducted in the sand hills of Kansas and Nebraska, as noted by BATES and PIERCE.[40] While the sand hills of Kansas are not extensive, almost a fourth of Nebraska is thus classified. Trees and even shrubs are not naturally very abundant in the sand hill region except along streams. In the planting a cue is taken from nature in the presence of *Pinus ponderosa* in the sand hill region; the occurrence of isolated tracts of this species suggests a former more extensive distribution. At the suggestion of Professor BESSEY, the Forest Service began planting as far back as 1891. About ten years ago, large tracts of land capable of forestation were set aside as national forests, and nurseries were established at Halsey, Nebraska, and Garden City, Kansas. In the Nebraska nursery, attention has been paid to conifers, and success has been had especially with *Pinus Banksiana* and the native *P. ponderosa*. In the Kansas nursery, experiment has been made chiefly with hardwoods.—H. C. COWLES.

The chemistry of symbiosis.—Not much is known concerning the exact chemical interrelations of symbionts. To ZELLNER it is a matter of surprise that investigators of symbiosis have paid so little attention to this fundamentally important feature, and he indicates in a brief paper[41] some of the places where more knowledge is urgently needed. Best known, of course, are the chemical interrelations existing between bacteria and Leguminosae. The significance of mycorhiza is much in dispute; ZELLNER'S view is that the fungi are water-absorbing organs for the roots. In the endotrophic forms phago-

[39] CANNON, W. A., A note on a chaparral-forest relation at Carmel, California. Plant World 16:36–38. 1913.

[40] BATES, C. G., and PIERCE, R. G., Forestation of the sand hills of Nebraska and Kansas. Bull. 121, U.S. Forest Service. pp. 49. *pls. 13. fig. 1.* 1913.

[41] ZELLNER, JULIUS, Die Symbiose der Pflanzen als chemisches Problem. Beih. Bot. Centralbl. 28[1]: 473–486. 1912.

cytosis is an important feature that should be looked into. As to lichens, it is noted that the chemistry of the plant complex is very different from the chemistry of the sum of the components, grown as separate individuals; the nutritive relations of the algal symbionts are not known. Similar suggestions are made relative to the need of investigating the chemistry of parasitism, as illustrated by ergot, wheat rust, and their host plants.—H. C. Cowles.

Lichens in relation to their substratum.—Bachmann,[42] who for many years has paid attention to the substratum relations of lichens, has reported his observations on the lichens of granite and quartz. Granite is decomposed into a claylike substance by lichen tissue with some rapidity, the micaceous constituents being particularly subject to ready decay. The quartz elements, on the other hand, are extremely resistant to such decomposition.

In a later paper Bachmann[43] reports the results of studies on calcareous lichens with *Chroolepus* gonidia. He finds that the *Chroolepus* itself is able to dissolve calcium carbonate, so that after a time a limestone becomes perforated in spongelike fashion through the agency of the *Chroolepus* cells of the fungal hyphae. As soon as the *Chroolepus* cells become inclosed by hyphae, they bud in a yeastlike manner and take on bizarre forms. On account of its position within a rock, such a lichen retains moisture longer than do ordinary superficial lichens.—H. C. Cowles.

Taxonomic notes.—Wernham[44] has described a new genus (*Neosabicea*) of Rubiaceae from Colombia. It belongs to the tribe Mussaendeae.

Dümmer[45] has described two new species of *Callitris*, one from New Caledonia and the other from the mountains of Ngoye.

Benedict[46] has begun a revision of the genus *Vittaria*. The first paper is a discussion of seven species, representing the subgenus *Radiovittaria*, and includes two new species.

Baker[47] has published a study of the African species of *Crotalaria*, preceding the descriptive list by a historical introduction, and also a discussion of the delimitation of the genus. The paper recognizes 309 species, the genus extending from Egypt and the Soudan and the Sahara to Cape Colony in the

[42] Bachmann, E., Die Beziehungen der Kieselflechten zu ihrer Unterlage. II. Granat und Quarz. Ber. Deutsch. Bot. Gesells. **29**:261–273. *figs. 4.* 1911.

[43] ———, Der Thallus der Kalkflechten. II. Flechten mit Chroolepugonidien. Ber. Deutsch. Bot. Gesells. **31**:3–12. *pl. 1.* 1913.

[44] Wernham, H. F., New Rubiaceae from tropical America. Jour. Botany **52**: 225–277. *pl. 533.* 1914.

[45] Dümmer, R. A., Three Conifers. Jour. Botany **52**:236–241. 1914.

[46] Benedict, R. C., A revision of the genus *Vittaria* J. E. Smith. Bull. Torr. Bot. Club **41**:391–410. *figs. 7. pls. 15–20.* 1914.

[47] Baker, E. G., The African species of *Crotalaria*. Linn. Soc. London. Bot. **42**:241–425. *pls. 9–14.* 1914.

south. In the list there are included descriptions of 76 new species and varieties.—J. M. C.

Flora of Shikotan.—TAKEDA[48] has studied somewhat intensively the flora of Shikotan, which is a small island situated near enough to the Kurile Islands to be regarded as one of them, at least in climatic conditions. The great interest of the islands in general is that the vegetation is quite primeval, nothing having been disturbed by the hand of man; in fact, Shikotan seems not to have been touched by human hands at all. An analysis of the floristic features is presented, and the enumeration includes 234 species, the largest assemblage being dicotyledons (219). The four largest families appear in the following order of abundance: Compositae, Gramineae, Rosaceae, and Umbelliferae. The largest genus is *Carex*, with 15 species; and 28 families are represented by a single genus, 23 of these genera being represented by a single species. The list includes the description of 5 new species.—J. M. C.

Phytogeographic notes from Palestine.—AARONSOHN[49] has called attention to some species that are disappearing from the flora of Palestine. He describes a little known station of *Acacia albida*, a species of northern Africa heretofore regarded as merely cultivated in Palestine. AARONSOHN regards it as an indigenous relict. Among other rare relicts in Palestine are *Pinus halepensis*, *Juniperus phoenicea*, and *Fraxinus oxycarpa oligophylla*. The author believes that these species, on account of the great need for wood in the arid Palestine climate, have been essentially exterminated by man. An interesting argument in support of this view, recalling the methods employed by the English ecologists in working out the original distribution of the beech, is based on the occurrence of place-names derived from these trees in neighborhoods where these species are no longer to be found.—H. C. COWLES.

U.S. Forest Service.—Among various articles of more or less general interest in a recent periodical, JAENICKE[50] gives a brief and interesting résumé of the varied activities of the Forest Service. This organization, employing the services of 2,895 persons, many of them with botanical training, and expending annually some $6,000,000, devotes its attention to subjects ranging from purely botanical research through reforestation and forest protection to the sale of timber and the development of water power. With increasing interest in forest protection, there is coming an increasing demand for increasing

[48] TAKEDA, H., The flora of the island of Shikotan. Jour. Linn. Soc. Bot. **42**: 433–510. 1914.

[49] AARONSOHN, A., Notules de phytogéographie palestinienne. (I). Une station peu connue de l'*Acacia albida* Del. (II). Espèces en voie d'extinction. Bull. Soc. Bot. France **60**:495–503, 585–592. *pl. 1.* 1913.

[50] JAENICKE, A. J., Progress of the U.S. Forest Service as reflected in the forester's reports of 1911, 1912, 1913. Forestry Quarterly **12**:397–407. 1914.

efficiency and more scientific knowledge of the principles underlying the various phases of forest administration, and these demands are being met as far as the limited funds permit.—GEO. D. FULLER.

Parasitic fungi of Wisconsin.—DAVIS[51] has brought together in a single list the parasitic fungi of Wisconsin reported in a succession of previous lists, beginning with that of A. F. BUNDY, published in the Report of the Geological Survey issued in 1873–1879, and including 30 species. The next list was that of TRELEASE (1884), and since then DAVIS has been indefatigable in adding species which justified the publication at intervals of supplementary lists. The final list contains 825 species of parasitic fungi and about 750 hosts. The Phycomycetes are represented by 61 species, 24 of which belong to *Peronospora*. The Ascomycetes number 502 species, the largest genus being *Septoria*, with 121 species. The Basidiomycetes number 256 species, all but 6 of which are smuts and rusts.—J. M. C.

Sand dune plants.—In a study of the flora of some sand dunes near the sea between Redonda and Venice, California, COUCH,[52] has made a floristic census of a number of quadrats, showing that in this area *Gaertneria bipinnatifida* is the dominant pioneer plant, but as the succession advances with increasing stability of the substratum, it is succeeded by *Abronia umbellata*, which is closely followed by *Eriogonum parvifolium*, *Adenostoma fasciculatum*, *Cheiranthus suffrutescens*, and *Lupinus Chamissonis*. Attention is also directed to the two kinds of competition here evident, that between the plants and their environment, and that between the plants themselves.—GEO. D. FULLER.

Antagonistic symbiosis in lichens.—TREBOUX'[53] studies of *Cystococcus humicola*, an alga that occurs free in nature and also in symbiosis with lichen fungi, lead him to the view that the lichen fungus is essentially parasitic. He concludes that the physiology of this alga is the same, whether inside or outside of a fungal symbiont; it does not require protein food (peptone) in either case, but can secure its nitrogen from nitrates or ammonium salts. Among the points in favor of the theory of parasitism are the smaller size of the symbiotic algae as compared with the free algae, less frequent cell division, diseased aspect where in contact with haustoria, and the relative absence of pyrenoid starch.—H. C. COWLES.

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[51] DAVIS, J. J., A provisional list of the parasitic fungi of Wisconsin. Trans. Wis. Acad. Sci. 17:846–984. 1914.

[52] COUCH, E. B., Notes on the ecology of sand dune plants. Plant World 17:204–209. 1914.

[53] TREBOUX, O., Die freilebende Alge und die Gonidie *Cystococcus humicola* in Bezug auf die Flechtensymbiose. Ber. Deutsch. Bot. Gesells. 30:69–80. 1912.

The vegetation of California.—CANNON[54] has published the address on the vegetation of California in relation to environment, which he delivered in 1913, at Carmel, California, before the members of the International Phytogeographic Excursion. The California environment is highly specialized, owing to the great climatic diversity, which in turn is associated with physiographic complexity. The corresponding specialization of the vegetation is shown in the marked vegetational types and also in the large display of endemism. An example of another sort of specialization is brought out in a consideration of the root relations of the oaks.—H. C. COWLES.

Branching of Rhizophora roots.—The repeated branching of the prop roots of *Rhizophora* is well known and has often been described. DOCTERS VAN LEEUWEN[55] has made the remarkable discovery that this branching is not a fixed feature of the roots, but is caused by an unidentified Scolytid beetle, which eats the growing portion of the roots. The destruction of a growing root tip is followed by the appearance of a lateral branch, about a centimeter above the killed portion. One plant was found far from the sea, in which an uninjured root grew down to the ground without branching.—H. C. COWLES.

Bees and cotton blossoms.—Stimulated by the discordant views as to the office of flower color in the attraction of insects to flowers, H. A. ALLARD[56] has made a series of observations on the visitation of cotton blossoms by bees, especially by *Melissodes*. It is concluded that the showiness of the flowers is the chief factor determining the insect visits. The removal or covering of the petals greatly reduces the number of visits. Only 12 per cent of the flowers inspected by bees were actually entered by them. Evidence is given of the influence of associative memory.—H. C. COWLES.

Polyporaceae of Ohio.—OVERHOLTS[57] has published a monograph on the Polyporaceae of Ohio, with full descriptions and keys. Approximately 100 species are described, representing 10 genera. One of the features of the monograph is that the descriptions are exactly comparable with one another, so that the contrasting characters are brought out with unusual clearness.—J. M. C.

[54] CANNON, W. A., Specialization in vegetation and in environment in California. Plant World 17:223–237. *figs. 3.* 1914.

[55] DOCTERS VAN LEEUWEN, W., Über die Ursache der wiederholten Verzweigung der Stützwurzeln von *Rhizophora*. Ber. Deutsch. Bot. Gesells. 29:476–478. *figs. 2.* 1911.

[56] ALLARD, H. A., Some experimental observations concerning the behavior of various bees in their visits to cotton blossoms. Amer. Naturalist 45:607–622, 668–685. 1911.

[57] OVERHOLTS, L. O., The Polyporaceae of Ohio. Ann. Mo. Bot. Garden 1:81–155. 1914.

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ADDITIONAL EVIDENCE OF MUTATION IN OENOTHERA[1]

HARLEY HARRIS BARTLETT

(WITH SEVENTEEN FIGURES)

Introduction

Much of the advance which has been made in genetics and practical breeding during the last decade has been a direct result of the promulgation by DE VRIES of the theory of the origin of species and varieties by mutation. That recessive Mendelian variations originate singly by mutation has been shown by several investigators, notably by MORGAN, who has observed the origin of more than 150 such variations in his cultures of *Drosophila*. Many opponents of the mutation theory deny, however, that progressive mutations ever occur in homozygous strains, or that true species, differing from the parent in several independent characters, have ever been observed to originate at a single step by mutation. DAVIS,[2] for example, is in accord with the mutationists in regarding *Oenothera gigas* as a marked progressive mutation of specific rank, but he denies that *Oenothera Lamarckiana*, the parent form of *O. gigas*, is homozygous. The facts (1) that *O. Lamarckiana* is not known as a native component of any flora, (2) that its known history has been that of a cultivated plant or an escape from cultivation,

[1] Published by permission of the Secretary of Agriculture.

[2] DAVIS, B. M., Cytological studies on *Oenothera*. III. A comparison of the reduction divisions of *Oenothera Lamarckiana* and *O. gigas*. Ann. Botany 25:941–974. 1911. "*Oenothera gigas* is a progressive mutant, its peculiarities being clearly associated with the changes in its germ plasm incident upon the doubling of its chromosome number" (*op. cit.* p. 974).

and (3) that its habit of throwing off marked germinal variations is paralleled by the behavior of certain interspecific hybrids in the F_2 and F_3 generations seem to DAVIS[3] a sufficient indication that this plant is of comparatively recent hybrid origin, and that its mutations are due to germinal instability resulting from hybridization. He holds that the germinal variations of *O. Lamarckiana* and of various hybrids which he has studied show marked progressive evolution which seemingly cannot be accounted for on a Mendelian basis. Although he does not deny that slight discontinuous variations may occur in homozygous strains (and he insists that the term mutation ought to be used only for such variations), he is of the opinion that variations large enough to be of evolutionary significance occur rarely if at all except in heterozygous lines.

GATES[4] does not believe that *O. Lamarckiana* is a recent interspecific hybrid, but does ascribe its mutations to germinal instability caused by occasional random crossing with other types. In their main conclusion, that when germinal variation occurs it usually follows crossing, DAVIS and GATES appear to agree. GATES, however, is more emphatic than DAVIS in his conclusion that mutation in *Oenothera* is not merely a result of Mendelian redistribution of unit characters, but is a distinct type of variation. He believes, moreover, that mutation sometimes takes place in pure as well as in hybrid lines. Since the phenomena are identical in the two cases, he has laid especial stress on the fact that there is no excuse for confusing mutation, when it occurs in hybrids, with any type of Mendelian segregation.[5]

[3] DAVIS, B. M., Genetical studies on *Oenothera*. II. Amer. Nat. 45:193–233. 1911; III. *Ibid.* 46:377–427. 1912; IV. *Ibid.* 47:449–476, 547–571. 1913.

[4] GATES, R. R., Mutation in *Oenothera*. Amer. Nat. 45:577–606. 1911.

———, A contribution to a knowledge of the mutating *Oenotheras*. Trans. Linn. Soc. Lond. II. Bot. 8:1–67. 1913.

———, Tetraploid mutants and chromosome mechanisms. Biol. Centralbl. 33:92–99, 113–150. 1913.

[5] In this author's last paper he says: "The cytological evidence shows that germinal changes may and do occur which are independent of all the laws of hybrid combination and hybrid splitting. This generalization is of more fundamental significance than might at first appear; for it shows that mutation in *Oenothera* is a process *sui generis*, and that no amount of hybrid combination and splitting, Mendelian or otherwise, is sufficient to account for it." GATES, R. R., Breeding experiments which show that hybridization and mutation are independent phenomena. Zeitschr. Ind. Abstammungs- u. Vererbungslehre 11:209–279. 1914.

HERIBERT-NILSSON[6] has made the first serious effort to explain the variations of *Oenothera Lamarckiana* on a strictly Mendelian basis. He does not hold with DAVIS that this species is necessarily of hybrid origin, but rather that it is a collective species, embracing a number of different strains which constantly cross among themselves. Consequently he assumes that the mutation phenomena do not exemplify progressive and regressive species formation, but merely the synthesis of new combinations from factors already existing within the species.

Although it is by no means true, as some critics seem to imply, that the mutation theory must stand or fall on the evidence derived from *Oenothera*, it must nevertheless be admitted that failure to find a parallel among other more fortunately chosen species of this genus to the mutation phenomena shown by *O. Lamarckiana* would discredit, if not invalidate, much of the direct evidence of mutation which has been so laboriously won by DE VRIES. DAVIS[7] has said that "it is evident that the adherents of the mutation theory are sensitive to the doubts freely expressed concerning the status of *Oenothera Lamarckiana*, the behavior of which in throwing off marked variants is cited as the most important evidence for the origin of species by mutations. . . . . Consequently, mutationists are likely to bring forward as rapidly as possible any evidence that may seem to indicate the appearance of clear inheritable variations of a marked character in forms of pure germinal constitution, i.e., in homozygous material."

It is the object of this paper to present additional evidence of mutation in *Oenothera*, derived from one of the small-flowered, self-pollinating wild American types. Before proceeding farther, however, it should be stated that a considerable body of similar evidence has already been obtained.

DE VRIES[8] and STOMPS[9] have twice observed the origin of a dwarf variety of *O. biennis* by mutation, once in a pure line of

[6] HERIBERT-NILSSON, N., Die Variabilität der *Oenothera Lamarckiana* und das Problem der Mutation. Zeitsch. Ind. Abst. u. Vererb. 8:89–231. 1912.

———, *Oenothera* Problemet. Svensk. Bot. Tidskr. 7[1]: pp. 16. 1913.

[7] DAVIS, B. M., Mutations in *Oenothera biennis* L? Amer. Nat. 47:116–121. 1913.

[8] DE VRIES, H., Die Mutationen in der Erblichkeitslehre. pp. 28–30. 1912.

———, Gruppenweise Artbildung. pp. 299–306. 1912.

[9] STOMPS, THEO. J., Mutation bei *Oenothera biennis* L. Biol. Centralbl. 32:521–535. 1912.

O. biennis var. *leptomeres*,[10] and once in a cross between this variety and typical *Oenothera biennis*, from which var. *leptomeres* itself doubtless arose by mutation. STOMPS has also described *O. biennis* mut. *semigigas* from the same culture of *O. biennis* var. *leptomeres*×*O. biennis* which gave rise to the dwarf. A recent letter from Professor DE VRIES (dated May 16, 1914) states that mutations from *O. biennis* are still being obtained at Amsterdam.

STOMPS[11] has just published a second report on mutations in *O. biennis*. He records the origin by mutation, in a pure line, of *O. biennis* var. *sulfurea* De V. (long known as a wild component of the Dutch flora), together with mut. *nanella* and mut. *semigigas*. GATES[12] has likewise announced the discovery of mutations (*O. biennis lata*, *O. biennis laevifolia*, *O. biennis rubrinervis*) from *O. biennis*, but has not yet published a full account of his cultures.[13] Finally, DE VRIES has obtained two different mutations, *O. salicifolia* and *O. salicastrum*, from wild seed of a strain of the self-pollinating *O. biennis* "Chicago" which he collected near Courtney, Missouri; and the writer[14] has given a preliminary account of *Oenothera stenomeres* mut. *lasiopetala*,[15] a hairy-petaled derivative of one of the small-flowered cruciate Onagras.

[10] *Oenothera biennis* var. *leptomeres* Bartlett. Amer. Jour. Bot. 1:242. 1914 = *Oenothera biennis* var. *cruciata* De Vries, not T. & G.

[11] STOMPS, THEO. J., Parallele Mutationen bei *Oenothera biennis* L. Ber. Deutsch. Bot. Gesells. 32:179–188. 1914.

[12] GATES, R. R., Parallel mutations in *Oenothera biennis*. Nature 89:659–660. 1912.

[13] Since the above was written, an account of the cytology of *O. biennis* mut. *lata* has been received. See GATES, R. R., and THOMAS, NESTA, A cytological study of *Oenothera* mut. *lata* and *O.* mut. *semilata* in relation to mutation. Quar. Jour. Micr. Sci. 59:523–571. 1914.

[14] BARTLETT, H. H., An account of the cruciate-flowered *Oenotheras* of the subgenus *Onagra*. Amer. Jour. Bot. 1:226–243. 1914.

[15] By an unfortunate oversight this name was published in Amer. Jour. Bot. as *O. stenopetala* mut. *lasiopetala*. The writer had originally used the name *O. stenopetala* for the species which was described as *O. stenomeres*. After the manuscript had been submitted to the editor, a change was made necessary by the publication of *O. stenopetala* Bicknell, Bull. Torr. Bot. Club 41:79. 1914. In one place the original name escaped notice and was not corrected. It is hoped that the error will not lead to any confusion.

It is shown in this paper (1) that the phenomena of mutation are as characteristic and as easily observed in one of the wild small-flowered self-pollinating Onagras as in *Oenothera Lamarckiana;* (2) that the mutations show characters unlike those of any other form with which the parent could have crossed; and (3) that the mutations cannot be ascribed to Mendelian segregation as at present understood. It therefore seems in the highest degree probable that mutation is a phenomenon which is independent of hybridization, and that the evidence of mutation which DE VRIES has found in *Oenothera Lamarckiana* is just as valid as though that species were known as a wild plant and not suspected of having had a horticultural origin.

Differential germination

Several of the most interesting mutations which were observed during the season of 1913 were found quite by chance. One lot of potting soil, in which the seeds of several strains were sown, proved to be a very stiff clay on which a hard crust formed. Germination was so poor that in several cases less than a dozen seedlings resulted from sowing perhaps a thousand or more seeds. It was afterward found that the seeds showed the usual percentage of germination when sown in good soil. In three different species the small progenies obtained when the seeds were planted under unfavorable conditions disclosed striking mutations, which had survived as a result of differential or selective germination. These mutations might easily have been overlooked in a seed pan containing several hundred seedlings, of which only a few were to be retained and grown to maturity.

The three mutant species were from widely separated localities. The seeds of one, from Plymouth, Massachusetts, were sent by Professor B. M. DAVIS; the others were collected by the writer at White Sulphur Springs, W.Va., and Lexington, Ky., respectively. The mutations of the two former species were lost before they matured. It will be useless, therefore, to give an account of their characters or of the cultures in which they appeared until they shall have been found again. In the case of the third species,

O. pratincola, the mutations were brought to maturity and have yielded a second generation. This species, therefore, has been systematically examined for variations, with the results recorded in this paper.

The Oenothera population at Lexington, Kentucky

During a brief visit in October 1912, the writer was able to find only two species of *Oenothera*×*Onagra* at Lexington, Ky. They are both new and are referred to below under the names *O. pratincola* and *O. numismatica*. If any other species occur within two or three miles of the city, they must be very scarce. Of course, in October many plants were through blooming and not in such condition that any differences among them would show to the best advantage. Nevertheless, it is believed that no common species could have been overlooked. Nine seed collections were made from individual plants, which showed as great a range of variation as possible. These plants, and the strains descended from them, have been designated by letters from A to I. Eight of the strains proved to be taxonomically identical and are referred to as *O. pratincola*. Lexington A, B, and C were collected in a pasture near Town Creek, 2 miles west of Lexington, where they grew within 200–300 feet of each other. Lexington E, F, G, H, and I were collected at random in vacant lots and within a mile of the city on the west. Lexington D is the only strain of the 9 which is referred to *O. numismatica*. The parent plant grew by a roadside about 2 miles east of Lexington. In addition to the seed collections, many rosettes were collected which flowered in Washington in 1913. Thirteen plants from the same general region as plants E to I proved on flowering to be typical *O. pratincola*, as were also 26 plants from the edge of a field near the reservoir east of the city. It thus appears that *O. pratincola* constitutes the bulk of the *Oenothera* population at Lexington. *O. numismatica* is much scarcer; it did not occur at all among the rosettes which were collected, and was seen in flower only east of Lexington.

The salient characters of the two evening primroses obtained at Lexington are the following:

In *O. pratincola*	In *O. numismatica*
A well-grown plant, is 1.5 m. high, and loosely branched.	A well-grown plant, is about 1 m. high, and densely branched.
The basal branches are frequently simple.	The basal branches bear tertiary branches and resemble the main stem.
The flowering time lasts about six weeks.	The flowering time lasts only about two weeks.
The lax terminal spike often becomes 5–6 dm. long (see fig. 1).	The dense terminal spike is about 2 dm. long in fruit (see fig. 2).
The lateral branches below the terminal spike are few in number and become 4–5 dm. long.	The lateral branches below the terminal spike are numerous and are seldom over 2 dm. long.
The lowest bracts of the upper lateral spikes are ovate, and grade upward to lanceolate.	The lowest bracts of the upper lateral spikes are nearly orbicular and grade upward through oblong to lanceolate (see fig. 2).
The calyx segments are so sparsely pilose as to appear practically glabrous.	The calyx segments are closely and finely pubescent.
The hairs of the calyx segments are about 1 mm. long, thick-walled, acute, with multicellular tuberculate bases.	The hairs of the calyx segments are less than 0.5 mm. long, and belong to two types: (1) an acute thick-walled type without tuberculate bases, and (2) a thin-walled, round-ended, clavate or cylindrical type.

Technical diagnoses of these two species, together with a discussion of their possible relationships, have been published elsewhere.[16] *O. pratincola* appears to be a frequent plant in the North Central States. *O. numismatica*, on the contrary, is known only from Lexington and may well be a local species, possibly derived by mutation from *O. pratincola*. Its close resemblance in certain characters to one of the mutations of *O. pratincola* is pointed out elsewhere in this paper.

The mutations of "Lexington C"

Seeds from four of the parent plants of *O. pratincola* which had been selected at Lexington were planted early in the spring of 1913.

[16] BARTLETT, H. H., Twelve elementary species of Onagra. Cybele Columbiana 1:37–56. 1914.

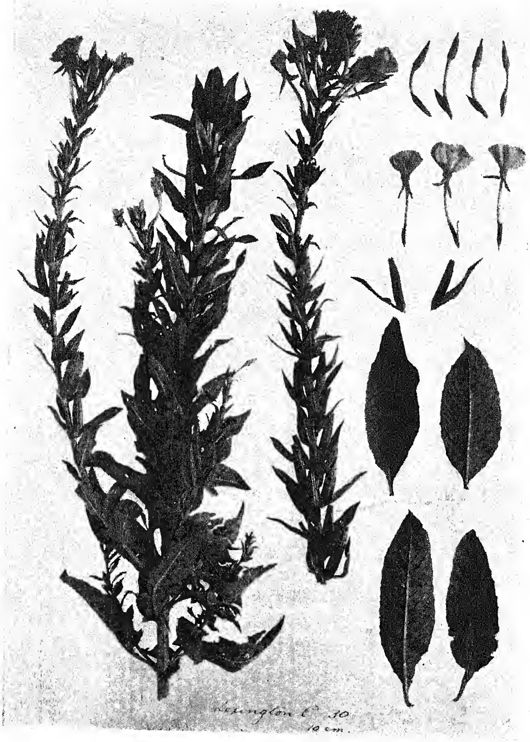

FIG. 1.—*Oenothera pratincola*, sp. nov.: upper part of main stem of Lexington C-30; leaves from middle of stem; flowers and buds; $\times\frac{1}{3}$.

FIG. 2.—*Oenothera numismatica*, sp. nov.: upper part of main stem of Lexington D-29; leaves from middle of stem; lateral branches from just below the terminal spike; characteristic foliage of such a lateral branch; in contrast with fig. 1, note the denser shorter spikes, which are only in flower a short time, the much closer branching, and the characteristic suborbicular leaves of the uppermost lateral branches; $\times\frac{1}{3}$.

Lexington A, B, and E germinated readily. Since no variation was noticed among the hundreds of seedlings of these three strains, all were discarded except 30 of each, which were potted off for the field cultures. The seeds of Lexington C, however, had been planted too deeply in unsuitable clay soil, and, although the seed

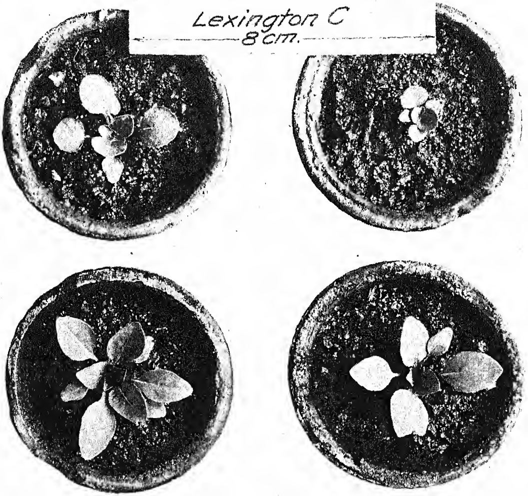

FIG. 3.—F_1 progeny of *Oenothera pratincola:* four of the 9 seedlings which constituted the first culture of Lexington C; the 2 upper plants, Lexington C-1 and C-2, are mut. *nummularia;* the latter bore seeds which gave rise to the F_1 culture referred to in table I; the 2 lower plants are typical *O. pratincola.*

pan received the same treatment as the rest, weeks passed before any seedlings appeared. At length 9 plants were obtained which were potted off. Almost from the first, they showed remarkable variation among themselves. Six (nos. 3, 4, 5, 6, 8, and 9) were typical *O. pratincola*, and agreed in all characters with the seed-

lings of Lexington A, B, and E; one (no. 7) was of a darker green color than the type, the leaves were somewhat narrower, and the petiole and midrib below the middle of the blade were particularly broad and white; two (nos. 1 and 2) had almost orbicular leaves, and constituted the most striking deviation from the expected form

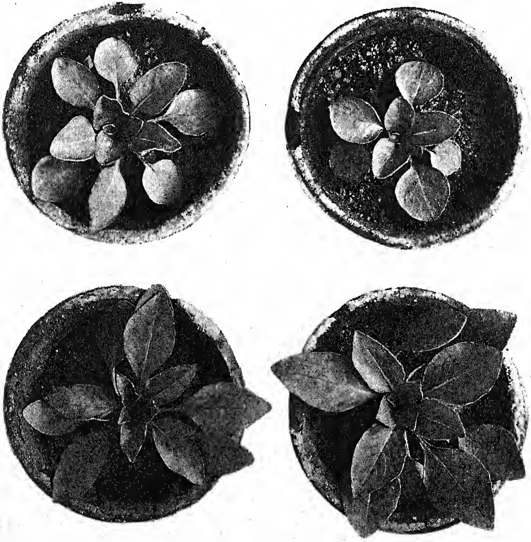

FIG. 4.—F_1 progeny of *Oenothera pratincola*, Lexington C: the same plants shown in fig. 3, but a month older.

that the writer had ever observed in a seedling of *Oenothera*. In fig. 3 the two upper plants are the round-leaved variations, nos. 1 and 2; the two lower are typical *O. pratincola*, nos. 3 and 4. In fig. 4 the same 4 plants are shown when a month older. The reader will observe that the orbicular seedling leaves of nos. 1 and 2 have

been superseded in the more mature rosette by leaves of a different form, but that the sharp distinction between the variation and the typical form has not been obscured. The occurrence of so interesting a variation in Lexington C led to a careful examination of the strain for evidence of mutability.

A second sowing of the same seed on good soil resulted in a progeny of 720 seedlings from 1000 seeds. As soon as the seedlings were well rooted, they were transplanted to square seed pans in which they were widely enough spaced to allow of unimpeded growth for a month or six weeks. This system was followed in all subsequent work. Of course, the seeds were invariably sown on sterilized soil. After the seedlings were transplanted, the pans were frequently examined for mutations, and all plants which were noticeably divergent from the mass of the culture were marked for preservation. Among the 720 seedlings of the second sowing, there were only 4 round-leaved plants. Since the mass of the culture was uniform, and the round-leaved plants constituted an absolutely discontinuous variation from both the typical form and one other pronounced variant which occurred in the culture, it was concluded that they were probably mutations. In the following pages the round-leaved type is called *O. pratincola* mut. *nummularia*.[17]

In order to show the discontinuity between typical *O. pratincola* and mut. *nummularia*, photographs of two of the seed pans in which this mutation occurred are reproduced as figs. 5 and 6. At the time the pans were photographed, the plants were about as far advanced as nos. 1–4 in fig. 3. Comparison of the figures will

[17] The writer has suggested (Amer. Jour. Bot. 1:237. 1914) that mutations of experimental origin be given trinomial names such as *O. pratincola* mut. *nummularia*, in order to avoid confusion with names which must be given consideration in floristic works. A trinomial nomenclature has the advantage over the binomial system proposed by GATES (Trans. Linn. Soc. London II. Bot. 8:10. 1913) in that the parallelism of mutations occurring in different species may be indicated by the use of the same mutational designation. For example, a convenient way to show the parallelism between the mutations of *O. Lamarckiana* and those of *O. biennis* would be to call them *O. Lamarckiana* mut. *semigigas*, *O. biennis* mut. *semigigas*, etc. The trinomial used in this way need imply nothing as to the specific, varietal, or formal rank of a mutation, but only the manner of its origin. Nevertheless, for the sake of avoiding confusion, it would be well not to give any mutation a name which had previously been used in any subspecific category within the species which had given rise to the mutation.

show the complete identity, at this stage of growth, of different individuals of mut. *nummularia*, and also the great uniformity of the typical plants of the culture.

FIG. 5.—F_1 progeny of *Oenothera pratincola*, Lexington C, pan 1: the only mutation shown is Lexington C-10, mut. *nummularia;* the other plants are typical *O. pratincola;* about the same age as the plants shown in fig. 3.

In addition to the 4 plants of mut. *nummularia* which were discovered in the second sowing, there were solitary specimens of each of two other mutations, one plant (no. 12) like no. 7 of the first planting, and another (no. 18) unlike anything else in the culture. No. 18 had exceedingly narrow, red, subulate seedling leaves and

was called on this account mut. *subulata*. There were also 7 plants (nos. 13–16, 19, 20, and 22) which developed very slowly and were retained in the expectation that they might prove to be dwarfs, although there was no character but size to distinguish

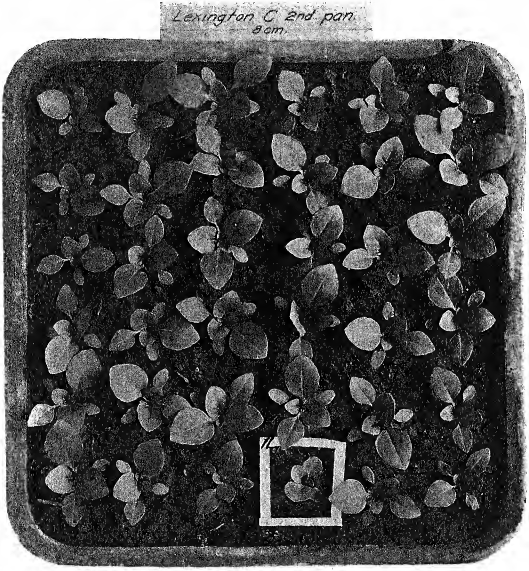

FIG. 6.—F_1 progeny of *Oenothera pratincola*, Lexington C, pan 2; the only mutation shown is Lexington C-11, mut. *nummularia;* the other plants are typical.

them from typical sister plants. The solitary plant of mut. *subulata* died, but the type has since been found to be one of the most frequent mutations of *O. pratincola*. The type represented by nos. 7 and 12 was designated as mut. *pusilla*. Its rosettes were about 4 cm. in diameter at maturity. The stem leaves were

linear-lanceolate. The stems were simple. No. 7 died just before flowering, when only 7 cm. high. No. 12 flowered at a height of 10 cm. The ovary was 7 mm. long; hypanthium 10 mm. long; calyx segments 4 mm. long, excluding the distant free tips, which were 1 mm. long. The calyx differed from that of the typical form not only in having distant calyx tips, but also in being densely soft-pubescent. Unfortunately, this plant was sterile and produced no seeds. As far as the writer is aware, mut. *pusilla* represents the extreme of nanism in the subgenus *Onagra*.

With a single exception, the 7 suspected dwarfs developed as quite normal plants, indistinguishable from the mass of the culture. One plant, no. 19, differed from the rest in that it had stiff, distant calyx tips 5 mm. long which were continued on the angles of the squarish bud as a marked carina. The buds were almost glabrous, as in the type form of the culture, but in marked contrast to some of the other mutations. This plant was self-sterile, but produced abundant seeds when pollinated with typical *O. pratincola*.

The Lexington C culture which was grown to maturity in 1913 included, besides the 9 plants from the first sowing and the mutations and suspected mutations of the second sowing, all the plants from two pans in which there appeared to be no variation. There were 72 of these plants, nos. 23–94.[18] When they matured two mutations were found which had not been detected in the early seedling stages. With these two exceptions, the plants were absolutely uniform among themselves, and exactly the same as Lexington A, Lexington B, and Lexington E. (Of each of these three strains 30 plants were grown to maturity.) The two mutations were not alike and were different from any of the other new types which had been obtained. Both, however, were almost

[18] The culture numbers of these plants are all given here in order to avoid lengthy repetition in subsequent papers which will deal with the same strains. It may be well to explain that every plant in the writer's garden is designated by the name of the strain (for which a number has often been substituted) followed by a succession of numbers which indicate the pedigree and number in the culture of each individual. Subscripts are used when it is wished to distinguish between sister plants grown in different years, or to indicate the years in which the successive generations were grown. "Lexington C-11_{13}," for example, would be the complete designation of the plant of mut. *nummularia* which is shown in fig. 6. Plants of the F_2 generation, grown in 1914, would be "Lexington C-11_{13}–1_{14}," "Lexington C-11–2," etc.

self-sterile. No. 28 was half as high as typical plants of the culture; the leaves were broader and white-margined; the buds were smaller and closely viscid-puberulent with a hair type which does not occur in the typical form; the branching differed in that there

FIG. 7.—A, *Oenothera pratincola* mut. *nummularia*, Lexington C-21; B, *Oenothera pratincola* (typical), Lexington C-16; the 2 plants are of the same age and were grown under identical conditions; note particularly the difference in the branching.

were numerous inflorescence-bearing tertiary branches. No. 57 (mut. *nitida*) was slightly taller than no. 28, almost simple, with leaves narrower than in the typical form, upwardly rolled parallel to the mid-vein, very glistening, nearly twice as thick as in normal

plants, and very brittle. A few seeds were obtained from this plant by self-pollination, but they did not germinate.

The 729 plants of Lexington C which were grown in 1913 yielded in all 6 different mutations. All of them except mut. *nitida* and mut. *nummularia* were infertile or nearly so to their own pollen. The latter type, in spite of the fact that it was unwittingly subjected to very unfavorable conditions, produced seeds from which a second generation has been grown. For fear of losing the 6 original plants, they were planted in the center bed of the greenhouse when the rest of the culture was transferred to the garden. Before it was realized how much their development was being retarded by the extreme heat in the greenhouse, it was too late to move them again with any prospect of success. Three plants died after they had begun to flower, but before any seeds were ripe.

The characters of mut. nummularia

A few plants of typical *O. pratincola* which were kept in the greenhouse with the mutation served to show that there are distinct differences in the habits of growth of the two types, when they are grown under identical conditions. This fact will be apparent from fig. 7, in which two sister plants of the same age are shown. It will be noticed that the stature of the mutation is less than that of the parent type, but that the lateral branches are more numerous and more densely leafy. A thoroughgoing comparison of the two types cannot be made until the cultures of 1914 shall have grown to maturity out of doors. The more striking contrasting characters, however, are the following:

In *O. pratincola*	In mut. *nummularia*
The early seedling leaves are ovate.	The early seedling leaves are orbicular.
The stem leaves are reflexed.	The stem leaves are involute.
The lower leaves of the lateral branches are ovate-lanceolate.	The lower leaves of the lateral branches are broadly ovate.
The ovary and calyx are sparsely pilose (sometimes almost glabrous).	The ovary and calyx are closely and finely pubescent.

In *O. pratincola*	In mut. *nummularia*
The hairs of the calyx segments and hypanthium are about 1 mm. long and all belong to the thick-walled, acute type with multicellular, tuberculate bases.	The hairs of the calyx segments and hypanthium are less than 0.5 mm. long and belong to two types: (1) acute, thick-walled hairs without multicellular, tuberculate bases, and (2) thin-walled, round-pointed clavate or cylindrical hairs.
The calyx segments separate in pairs.	The four calyx segments remain united when the flower opens.

The difference in the rupture of the calyx is shown in fig. 8. The writer is inclined to believe that the clear-cut qualitative distinction between the calyx pubescence of the parent form and that of the mutation will provide an absolute criterion for determining whether or not mut. *nummularia* marks an evolutionary advance over *O. pratincola*. A priori it seems to be a safe prediction that mut. *nummularia* will prove to be a progressive mutation of even more striking individuality than *O. gigas*.

It is unfortunate that data on reciprocal crosses between *O. pratincola* and mut. *nummularia* will not be available until next year. The first flowers of the original mutations were, of course, self-pollinated, and further work was prevented by the loss of the plants. This year (1914) the writer has numerous plants of mut. *nummularia* (primary mutations as well as F_1 plants) with which to make the necessary crosses.

The heritability of mut. nummularia

The three individuals of mut. *nummularia* which bore seeds were nos. 2, 17, and 21. Even these, however, wilted and dried up while still in flower, so that very few capsules were obtained. As in the case of many somewhat self-sterile Oenotheras, the capsules were small and contained few good seeds. From each of several capsules only one or two seeds were obtained, and the best had but 30, whereas a large capsule of typical *O. pratincola* contains well over 300. Until plants of the mutation shall have developed under more favorable conditions than those to which the first season's plants were subjected, it will be impossible to say

whether or not mut. *nummularia* is really as nearly self-sterile as this comparison would indicate. At any rate, only 403 seeds, many of them obviously too unripe to germinate, were obtained from 3 plants of the mutation. The seeds have given an F_1 progeny of 135 plants which is now (April 1914) in the early seedling stage.

The F_1 generation from mut. *nummularia* consists in part of plants which exactly reproduce the parental type and in part of secondary mutations. At the time this article is being written the plants are still young, but it is nevertheless clear (1) that the

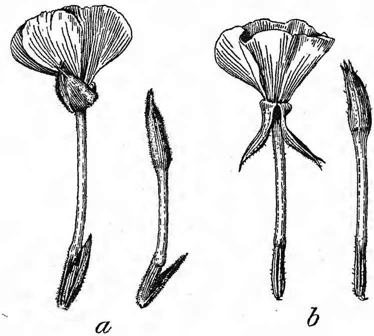

FIG. 8.—Flowers and buds of (*a*) *Oenothera pratincola* mut. *nummularia*, and (*b*) typical *O. pratincola*, showing especially the difference in the rupture of the calyx.

F_1 generation includes no typical *O. pratincola;* (2) that the secondary mutations (with one possible exception) are narrower leaved than *O. pratincola* and therefore even more sharply distinguished from mut. *nummularia* than the latter is from *O. pratincola;* and (3) that all of the secondary mutations (again with the single exception noted above) appear to be quite identical with certain primary mutations which have appeared simultaneously with mut. *nummularia* in various cultures of typical *O. pratincola*.

The secondary mutations fall into three well marked groups which have been called mut. *tortuosa*, mut. *rubricentra*, and mut.

subulata. It is of course impossible to establish absolute identities among seedling plants of types which have not yet been seen in flower. Consequently the F_1 progeny of mut. *nummularia* are classified either as true to type or as secondary mutations in table I, which shows the composition of the cultures now under observation.

TABLE I

COMPOSITION OF F_1 GENERATION OF MUT. *nummularia* (SEEDLING STAGE)

Parent	Number of seeds	Total plants	Mut. *nummularia*	Secondary mutations
Lex. C-2	15*	8	6	2
"	15*	6	5	1
"	30*	15	15	0
"	65	9	7	2
Lex. C-2 Total	125	38	33	5
Lex. C-17	39	14	12	2
Lex. C-17×C-21	20*	10	8	2
"	24*	2	2	0
"	11*	5	3	2
"	18*	5	5	0
"	64	25	15	10
Lex. C-17×C-21 Total . . .	137	47	33	14
Lex. C-21	102	36	31	5
Grand total	403	135	109	26

* Indicates that the seeds were from one capsule.

Table I shows that only 34 per cent of the seeds of mut. *nummularia* germinated. In order to obtain as many plants as possible, a large number of seeds were counted into the seed pans which seemed too immature to germinate; 65 such seeds, planted by themselves, produced 9 plants. Part of the seeds planted were obtained from self-pollinated capsules, others from capsules which had been cross-pollinated. Table I shows that the progeny from the self-pollinated seeds includes secondary mutations and typical *nummularia* plants in the ratio 1:6. The same ratio for the progeny from cross-pollinated seeds is about 1:3.2. Although the difference in the ratio seems very marked, it may be due to the fact that the germination was poor and the cultures small.

Some of the progeny of two of the parents from which F_1 plants were obtained (Lexington C-17 and Lexington C-21) are shown in figs. 9–11. In fig. 9, no. 3 is a young specimen of mut. *tortuosa*, as yet only vaguely suggesting the characters which give this mutation its name. The other 5 plants are typical mut. *nummularia*, comparable in state of development with nos. 1 and 2 in fig. 3, and nos. 10 and 11 in figs. 5 and 6. Fig. 10 shows three of the types which are included in the F_1 cultures from mut. *nummularia*. Nos. 8 and 15 are characteristic plants of mut. *tortuosa;* nos. 13 and 16 are mut. *rubricentra;* nos. 35 and 36 are typical mut. *nummularia*. In order to show the striking uniformity of the *nummularia* plants 6 more of them are shown in fig. 11.

The frequency of mut. nummularia

In order to determine the frequency with which *O. pratincola* gives rise to mut. *nummularia*, large cultures were grown in the greenhouse during the winter of 1913–14. As usual, the seeds were sown on sterilized soil and transplanted to seed pans as soon after germination as circumstances permitted.[19] Remaining wild seeds of the original collections gave additional F_1 cultures of Lexington C, A, B, and E. F_1 cultures were also grown from the wild seeds of Lexington F, G, H, and I, which had not been previously planted. It will be remembered that mutations had been detected during the first year of cultivation only in Lexington C, and in this strain only because of the accidental application of the method of selective germination. The other strains were found to be quite as mutable as Lexington C when all of the seedlings were retained until old enough to show their distinctive characteristics. In addition to the F_1 cultures, F_2 cultures were grown from seeds of 8 self-pollinated F_1 sister plants of Lexington C, 1 self-pollinated plant of Lexington A, and 2 self-pollinated plants of Lexington B. These F_2 progenies from guarded seeds were found to contain approximately the same proportion of mutations as the F_1 progenies from unguarded wild seeds.

[19] The writer wishes to express here his appreciation of Mr. MARTIN BILON's painstaking and efficient care of the germination pans and the young seedlings.

FIG. 9.—F_1 progeny of *Oenothera pratincola* mut. *nummularia*, Lexington C-17; the plant in the upper left-hand corner, Lexington C-17–3, is *O. pratincola* mut. *tortuosa*, here occurring as a secondary mutation, but seemingly the same as one of the very rarest primary mutations of *O. pratincola;* the other plants are typical examples of mut. *nummularia*.

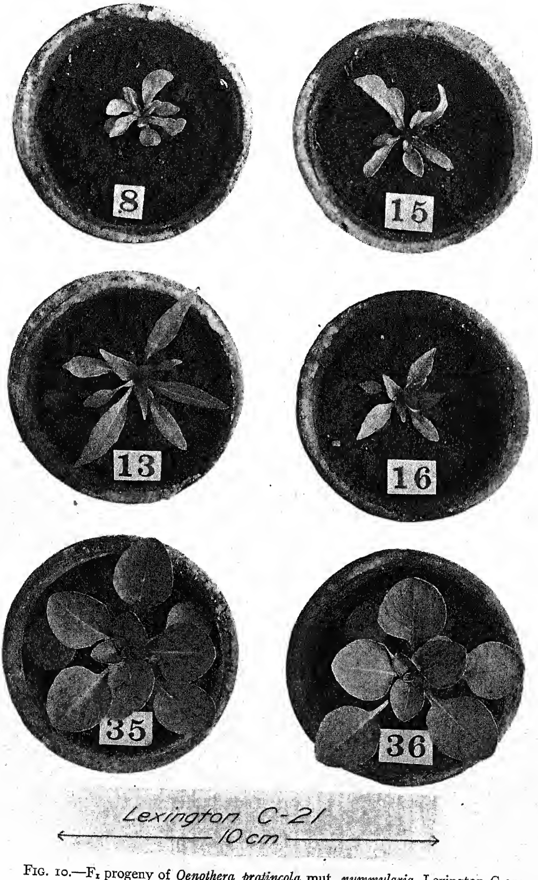

FIG. 10.—F_1 progeny of *Oenothera pratincola* mut. *nummularia*, Lexington C-21; the 2 upper plants, Lexington C-21–8 and C-21–15, are mut. *tortuosa;* the 2 in the middle row, C-21–13 and C-21–16, are mut. *rubricentra;* the 2 below are mut. *nummularia;* the plants shown in this cut are three weeks

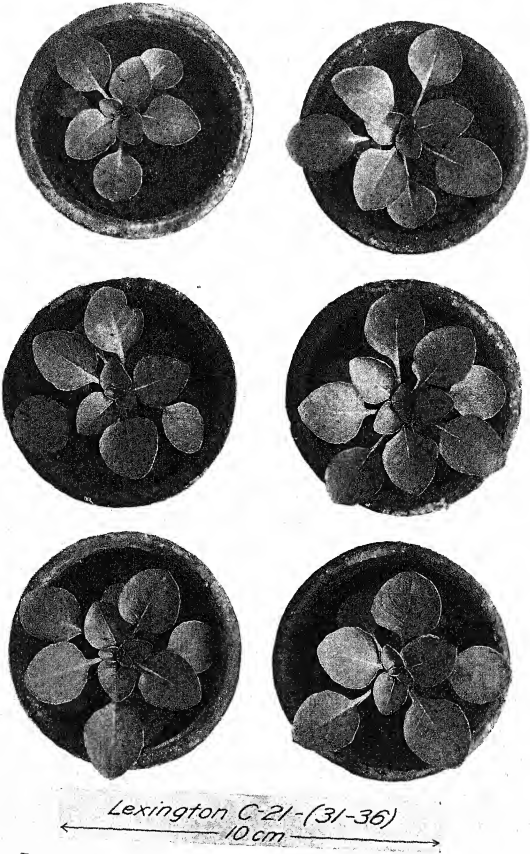

FIG. 11.—F_1 progeny of *Oenothera pratincola* mut. *nummularia;* typical examples of mut. *nummularia*, sister plants of those shown in fig. 10, of the same age.

Data in regard to the frequency of mut. *nummularia* in all the strains of *O. pratincola* except Lexington E are given in tables II–XI, and are summarized in table XII. Lexington E yielded striking mutations in both F_1 and F_2 generations, but they constituted an entirely different series of forms from those which were obtained from the other strains. In several respects the mutation phenomena presented by Lexington E were unique. It will be necessary, therefore, to defer an account of this strain until next year.

It will be noticed from the tables that a large number of seeds were planted capsule by capsule. The variation in number of seeds per capsule appears greater than it should, for in many cases the capsules had dehisced and lost part of their contents. In general, a capsule of *O. pratincola* contains 200–300 seeds.

TABLE II

ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON C"

Culture	Seeds planted	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
2	1000	720	711	4 (nos. 10, 11, 17, and 21)	5
3	133*	101	93	0	8
4	130*	117	116	0	1
5	116*	111	106	0	5
6	88*	21	20	0	1
7	125*	20	20	0	0
8	162*	152	149	0	3
9	164*	143	140	0	3
10	237*	172	165	0	7
11	237*	223	211	1 (no. 123)	11
12	65*	35	35	0	0
13	217*	147	143	1 (no. 136)	3
14	200	156	153	0	3
15	200	154	152	0	2
16	147*	96	93	0	3
17	200	60	58	0	2
18	200	155	152	2 (nos. 150 and 151	1
19	200	98	95	0	3
20	200	130	124	2 (nos. 156 and 160)	4
21	200	112	109	1 (no. 162)	2
Total	4,221	2,923	2,845	11	67

* Indicates seeds from the same capsule.

TABLE III

ANALYSIS OF F_2 SEEDLING CULTURES OF "LEXINGTON C"

Parent	Culture	Number of seeds	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
Lex. C-5	1	244*	151	150	0	1
"	2	208*	155	154	0	1
"	3	190*	140	138	1 (no. 3)	1
Lex. C-5	Total	642	446	442	1	3
Lex. C-6	1	116*	41	39	0	2
"	2	106*	41	40	0	1
"	3	58*	31	27	0	4
"	4	41*	35	35	0	0
"	5	21*	10	10	0	0
"	6	14*	13	13	0	0
Lex. C-6	Total	356	171	164	0	7
Lex. C-15	1	196*	116	115	1 (no. 1)	0
"	2	233*	115	115	0	0
"	3	173*	106	106	0	0
"	4	134*	123	122	1 (no. 2)	0
"	5	28*	12	12	0	0
"	6	31*	12	12	0	0
"	7	48*	41	41	0	0
"	8	43*	32	31	1 (no. 11)	0
"	9	12*	9	9	0	0
"	10	17*	15	15	0	0
"	11	31*	15	15	0	0
Lex. C-15	Total	946	596	593	3	0
Lex. C-22	1	148*	122	119	0	3
"	2	203*	169	157	1 (no. 9)	11
"	3	172*	147	142	1 (no. 20)	4
"	4	225*	189	184	1 (no. 21)	4
"	5	250*	196	191	0	5
Lex. C-22	Total	998	823	793	3	27
Lex. C-36	1	182*	142	141	0	1
"	2	208*	150	147	0	3
"	3	210*	159	157	0	2
"	4	231	181	179	0	2
"	5	147*	99	96	0	3
Lex. C-36	Total	978	731	720	0	11
Lex. C-52	1	215*	148	146	1 (no. 1)	1
"	2	118*	100	100	0	0
"	2	189*	138	136	0	2
"	4	150*	75	73	1 (no. 28)	1
"	5	225*	173	173	0	0
Lex. C-52	Total	897	634	628	2	4

TABLE III—*Continued*

Parent	Culture	Number of seeds	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
Lex. C-72	1	192*	171	165	1 (no. 6)	5
"	2	223*	180	179	0	1
"	3	235*	187	186	0	1
"	4	279*	242	241	0	1
"	5	280*	230	225	0	5
Lex. C-72	Total	1,209	1,010	996	1	13
Lex. C-91	1	185*	146	144	2 (nos. 1 and 2)	0
"	2	284*	245	243	1 (no. 4)	1
"	3	245*	172	171	0	1
"	4	267*	125	121	0	4
"	5	316*	198	195	0	3
"	6	337*	300	293	5 (nos. 39, 40, 42, 43, 44)	2
"	7	242*	187	181	0	6
Lex. C-91	Total	1,876	1,373	1,348	8	17
Eight F_1 plants	Grand total	7,902	5,784	5,684	18	82

* Indicates seeds from the same capsule.

TABLE IV

ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON A"

Culture	Seeds planted	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
2	341*	66	59	0	7
3	405*	129	118	2 (nos. 44 and 45)	9
4	337*	60	53	2 (nos. 53 and 54)	5
Total	1,083	255	230	4	21

* Indicates seeds from the same capsule.

TABLE V

ANALYSIS OF F_2 SEEDLING CULTURES OF "LEXINGTON A"

Parent	Culture	Number of seeds	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
Lex. A-5	1	119*	76	73	0	3
"	2	200	115	110	0	5
"	3	200	118	115	2 (nos. 9 and 11)	1
"	4	200	127	122	1 (no. 15)	4
Lex. A-5	Total	719	336	420	3	13

* Indicates seeds from the same capsule.

TABLE VI

ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON B"

Culture	Seeds planted	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
2	149*	107	95	0	12
3	324*	188	175	0	13
4	247*	107	97	0	10
5	200*	106	101	0	5
Total	920	508	468	0	40†

* Indicates seeds from the same capsule.

† Of the 40 mutations and suspected mutations, 36 were merely smaller plants than the average, selected in the expectation that some might prove to be dwarfs.

TABLE VII

ANALYSIS OF F_2 SEEDLING CULTURES OF "LEXINGTON B"

Parent	Culture	Number of seeds	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
Lex. B-1	1	184*	157	157	0	0
"	2	269*	142	137	0	5
"	3	264	221	215	1 (no. 8)	5
"	4	195*	154	152	1 (no. 12)	1
Lex. B-1	Total	912	674	661	2	11
Lex. B-2	1	106*	72	68	0	4
"	2	250*	147	142	2 (nos. 26, 27)	3
"	3	284*	176	171	0	5
"	4	111*	82	80	0	2
"	5	113*	94	92	0	2
Lex. B-2	Total	864	571	553	2	16
Two F_1 plants	Grand total	1,776	1,245	1,214	4	27

* Indicates seeds from the same capsule.

TABLE VIII

ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON F"

Culture	Seeds planted	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
1	92* (large, immature)	68	62	1 (no. 1)	5
2	139* (mature)	34	31	0	3
Total	231	102	93	1	8

* Indicates seeds from the same capsule.

TABLE IX

ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON G"

Culture	Seeds planted	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
1	87* (very large, immature)	25	25	0	0
2	50* (large, immature)	17	17	0	0
3	285*	157	152	0	5
4	160	140	139	0	1
5	159*	136	133	2 (nos. 47, 49)	1
6	152*	95	93	0	2
7	187*	153	152	0	1
8	144*	86	80	0	6
9	120*	106	99	0	7
10	197*	157	154	1 (no. 54)	2
11	133*	98	97	0	1
12	147*	114	110	1 (no. 52)	3
Total	1,821	1,284	1,251	4	29

* Indicates seeds from the same capsule.

TABLE X

ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON H"

Culture	Seeds planted	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
1	301*	152	145	2 (nos. 3 and 4)	5
2	157*	78	76	0	2
Total	458	230	221	2	7

* Indicates seeds from the same capsule.

TABLE XI

ANALYSIS OF F_1 SEEDLING CULTURES OF "LEXINGTON I"

Culture	Seeds planted	Total plants	Typical	Mut. *nummularia*	Other muts. and suspected muts.
1	125*	57	53	0	4
2	266*	147	145	1 (no. 13)	1
3	244* (immature)	64	61	2 (nos. 14 and 15)	1
Total ...	635	268	259	3	6

* Indicates seeds from the same capsule.

TABLE XII

SUMMARY OF TABLES II–XI, SHOWING THE FREQUENCY WITH WHICH *O. pratincola* GIVES RISE TO MUT. *nummularia*

Strain	Generation	Number of seeds	Number of plants	Germination per cent	Number of mut. *nummularia*	Ratio of mut. *nummularia* to seeds planted	Ratio of mut. *nummularia* to total plants
Lex. A ..	F_1	1083	255	23.5	4	1:271	1:64
" ..	F_2	719	436	60.6	3	1:240	1:145
Lex. A ..	F_1 & F_2	1,802	691	38.4	7	1:257	1:99
Lex. B ..	F_1	920	508	55.2	0		
" ..	F_2	1776	1245	70.1	4	1:444	1:311
Lex. B ..	F_1 & F_2	2,696	1,753	65.0	4	1:674	1:438
Lex. C ..	F_1	4221	2923	69.3	11	1:384	1:266
" ..	F_2	7902	5784	73.2	18	1:439	1:321
Lex. C ..	F_1 & F_2	12,123	8,707	71.8	29	1:418	1:300
Lex. F ..	F_1	231	102	44.2	1	1:231	1:102
Lex. G ..	F_1	1,821	1,284	70.5	4	1:455	1:321
Lex. H ..	F_1	458	230	50.2	2	1:226	1:115
Lex. I...	F_1	635	268	42.2	3	1:212	1:89
All.....	F_1	9,369	5,570	59.5	25	1:375	1:223
"	F_2	10,397	7,465	71.8	25	1:416	1:299
All.....	F_1 & F_2	19,766	13,035	66.0	50	1:395	1:261

In all, there were 19,766 seeds sown of the 7 strains which gave rise to mut. *nummularia*. They gave 13,035 seedlings, of which 5,570 belonged to F_1 and 7,465 to F_2 progenies. The average germination of the F_1 seeds was 59.5 per cent, or 58 per cent if the 1000 seeds of Lexington C sown in the winter of 1912–1913 are not figured in. Most of the F_1 seeds were over a year old when they were planted. The germination of the F_2 seeds, which were sown soon after they were harvested, was 71.8 per cent. Inspection of table XII shows the remarkable fact that the ratio of mut. *nummularia* to seeds planted was nearly identical for the F_1 and F_2 progenies, 1:375 in the one case, 1:416 in the other, but that the

ratio of *nummularia* mutations to plants showed a variation roughly commensurate with the difference in germinability between the F_1 and F_2 seeds. In other words, the mortality among the year-old F_1 seeds appears to have been largely confined to seeds of typical *O. pratincola*. The ratio of *nummularia* mutations to seeds planted is seen from table XII to be reasonably constant for all 7 strains in both the F_1 and F_2 generations. The ratio of mutations to total plants, however, varies between wide limits, and in every case a low percentage of germination is associated with a high frequency of mutation. The F_1 progeny of Lexington A, for example, included 4 individuals of mut. *nummularia* among 255 plants, a ratio of 1:64. These 255 plants, however, were obtained by sowing 1,083 seeds, of which only a small proportion (23.5 per cent) germinated. There seems no escape from the conclusion that the percentage of germinable seeds of mut. *nummularia* had increased by virtue of the greater mortality among the seeds of typical *O. pratincola*.

The evolutionary significance of differential mortality is too obvious to require any lengthy discussion. Mut. *nummularia* has a distinctly greater survival value than its parent when subjected to conditions which delay germination. It has already been shown that mut. *nummularia* has an enormously greater chance to survive than typical *O. pratincola* when subjected to certain unfavorable soil conditions. These facts should be carefully weighed by critics of the mutation theory who persist in assuming, as a matter of course, that mutations would have no chance to survive in competition with the more numerous typical plants. De Vries's[20] has already shown that the percentage of mutation in a culture of *O. Lamarckiana* from seeds 5 years old was 40 per cent instead of the usual 6 per cent. In his comment on this remarkable result he states that in general the seeds of the mutation remain germinable longer than those of typical *O. Lamarckiana*, and suggests that it might be possible to make use of differential mortality to increase the proportion of mutations in seeds, and thereby to facilitate the discovery of the mutations. The writer unconsciously put this suggestion to a test at the time mutations

[20] De Vries, H., Die Mutationstheorie 1:186. 1901.

were first found in *O. pratincola.* HUNGER[21] has recently recorded observations on selective mortality in the seeds of *O. Lamarckiana* which can only be interpreted as showing that the mutations of this species have decidedly a greater survival value than the parent form.

It is often remarked that the Onagras are not most usually found in undisturbed habitats with other native plants, but rather as weeds in fields and waste places, among the aliens of our flora. Wherever the soil is disturbed, as by plowing, road-making, excavating, they are frequently found in large numbers. They often dominate the flora on made land and on new railroad embankments, but are for the most part replaced by other weeds when the soil ceases to be disturbed at intervals. A fallow field which contains many Onagras for a season or two after cultivation is discontinued will thereafter contain fewer each year. If again plowed, it will apparently be restocked by the germination of seeds which have lain dormant, perhaps for years. Selective mortality among dormant seeds might result in such a field being restocked with plants among which mutations would be unexpectedly numerous.

The most interesting fact shown by table XII is that the frequency of mut. *nummularia* cannot correspond with any Mendelian ratio except that of a tetrahybrid splitting in the ratio 255:1. In the case of a number of progenies, to be sure, the ratio of mutations to plants more nearly approximates the trihybrid ratio 63:1, but it has already been shown that in each such instance the high mutation ratio is associated with a low percentage of germination. When the ratio of mutations to seeds is dealt with, there is no case of an approximation to the 63:1 ratio. The data of table XII, recalculated, are stated in table XIII in such form as to show that no single progeny was large enough to prove that the 255:1 ratio might not be the true one. On the contrary, the data afford no reason to believe that the mutation ratio is 255:1. It may be because of the smallness of the cultures that no single progeny shows a significant deviation from this ratio.

[21] HUNGER, F. W. T., Recherches expérimentales sur la mutation chez *Oenothera Lamarckiana*, executées sous les tropiques. Ann. Jard. Buitenzorg 27:92–113. 1913.

Turning again to table XII, it is seen that the progenies might possibly be assembled in two groups, those with a mutation ratio of approximately 400:1 (group I of table XIII), and those with a ratio of about 250:1 (group II of table XIII). Testing separately the ratios from these groups (see table XIII), we find that the number of individuals in group II is too small to establish a significant deviation from the ratio for group I. (The difference is 0.18±0.13 per cent). It is therefore impossible to demonstrate either that the mutation ratio is or that it is not the same for all the progenies.

. TABLE XIII

TEST OF THE FITNESS OF THE MUTATION RATIOS TO THE NEAREST MENDELIAN RATIO (235:1)

Culture	Group	Class 0 per cent not mut. *nummularia*	Class 1 per cent mut. *nummularia*	Number of seeds planted (n.)	Standard deviation $(\sigma=\sqrt{\%p_0 \cdot \%p_1})$	Mean error in per cent $\left(m=\frac{\sigma}{\sqrt{n}}\right)$	Expectation for each value of n if ratio is 255:1	Difference between observation and expectation
Lex. A-F_1	II	99.63	0.37	1,083	6.07	0.18	0.39±0.19	0.02±0.26
Lex. A-F_2	II	99.58	0.42	719	6.47	0.24	0.39±0.24	0.03±0.34
Lex. B-F_2	I	99.77	0.23	1,776	4.79	0.11	0.39±0.15	0.16±0.29
Lex. C-F_1	I	99.74	0.26	4,221	5.10	0.08	0.39±0.10	0.13±0.13
Lex. C-F_2	I	99.77	0.23	7,902	4.79	0.05	0.39±0.07	0.16±0.09
Lex. F-F_1	II	99.57	0.43	231	5.42	0.42	0.39±0.41	0.04±0.59
Lex. G-F_1	I	99.78	0.22	1,821	4.68	0.11	0.39±0.15	0.17±0.29
Lex. H-F_1	II	99.56	0.44	458	6.59	0.31	0.39±0.29	0.05±0.43
Lex. I-F_1	II	99.53	0.47	635	6.86	0.36	0.39±0.25	0.08±0.44
Group I		99.76	0.24	15,720	4.89	0.04	0.39±0.05	0.15±0.06
Group II		99.58	0.42	3,126	6.47	0.12	0.39±0.11	0.03±0.16
Groups I & II		99.75	0.25	18,846	5.00	0.04	0.39±0.05	0.14±0.06
Total		99.75	0.25	19,766	5.00	0.04	0.39±0.04	0.14±0.06

If we assume that it is justifiable to treat all of the progenies as one group, the numbers are then large enough to indicate, not however without considerable doubt, that the frequency of occurrence of mut. *nummularia* is not in accord with the tetrahybrid ratio 255:1, but with some ratio lying between 330:1 and 450:1. Of course we cannot assume that there is no mortality at all among the seeds which produce mut. *nummularia*. If in the 30 per cent of seeds *O. pratincola* which never germinate even when fresh the mortality among mutations and non-mutations were the same, then the mutation ratio would not significantly deviate from 255:1. It

is obvious that a Mendelian explanation of the occurrence and frequency of mut. *nummularia* involves the assumption that each parent plant which gave rise to it was heterozygous with regard to at least four factors. Otherwise no segregate would occur with so low a frequency as 1:255. The following objections to a Mendelian explanation may be enumerated:

1. *O. pratincola* is probably almost invariably self-pollinated in a state of nature, for the anthers burst in contact with the receptive stigma the day before the flowers open. In a very few generations heterozygosis would be eliminated from a strain which had accidentally become crossed. Hybridization involving four factors, followed by several generations of self-pollination, would result in an F_2 with 6.25 per cent of homozygotes, an F_3 with 31.64 per cent, F_4 with 58.62 per cent, F_5 with 93.75 per cent, F_6 with 96.87 per cent, F_7 with 98.44 per cent, F_8 with 99.22 per cent, F_9 with 99.61 per cent, F_{10} with 99.80 per cent, etc. It would be utterly absurd to suggest that out of 8 wild mother plants growing far apart, selected at random, 7 were tetrahybrids.

2. An F_1 tetrahybrid would invariably show segregation in a 255:1 ratio. Out of its F_2 progeny, however, only one plant in 16 would be a tetrahybrid, and therefore only one F_2 plant in 16 could exhibit 255:1 segregation in the F_3. The other F_2 heterozygotes would be hybrids of a lower order. Some would segregate in the ratio 63:1, some in the ratio 15:1, and some in the ratio 3:1. It has already been pointed out (see tables III, V, and VII, summarized in tables XII and XIII) that every F_1 (that is, F_1 with regard to the wild mother plants from Lexington) plant of which seeds were planted either yielded a progeny containing no *nummularia* mutations, in which case the number was not large enough to be sure of getting this mutation, or else the only Mendelian ratio indicated as possible was 255:1. In all, 11 F_2 progenies were grown, of which only 2 failed to give the mutation. The only uncomplicated Mendelian explanation requires that in picking 11 mother plants at random from among 142 F_1 plants, 9 were selected from that one-sixteenth of the culture which was still heterozygous for four characters. It may be pointed out that among 142 plants, just 9 tetrahybrids might reasonably be expected. The chances

are infinitesimal that all 9 would be included among 11 plants chosen at random.

3. A tetrahybrid might give as many as 16 phaenotypes in the F_2. All of these would have a greater frequency than 1:255 except the pure recessive. We have seen that mut. *nummularia* cannot have a greater frequency than 1:255, and have also seen that it is not a pure recessive, for in the next generation after it originates it gives rise to several distinct types.

4. In the case of one F_2 progeny (Lexington C-91, see table III) from a single mother plant, 1,539 seeds from 6 capsules gave 3 specimens of mut. *nummularia*, whereas 337 seeds from one capsule gave 5. Such a result shows a frequency varying from 1:60 to 1:513 on capsules from the same spike. From a Mendelian standpoint it is practically impossible to explain such a result.

The mutation phenomenon in *O. pratincola* cannot be explained away by any reasonably plausible stretching of Mendelian theory. On the contrary, it seems obvious that mutation is quite a different process from hybrid segregation, although both processes may occur simultaneously.

Mut. *nummularia* is the only one of the mutations of *O. pratincola* the frequency of which has been determined. None of the others has been observed throughout the complete cycle from seed to seed and carried into a second generation. In tables II–XI all of the variants except mut. *nummularia* are thrown together as "other mutations or suspected mutations." In explanation of this mixed category, it is necessary to state that all unusually small or unusually large plants, regardless of whether or not they appeared otherwise different from the mass of the culture, were counted as "suspected mutations," in order to be sure that no such variation as a mut. *nanella* would be passed over. To judge from past experience, most of the "suspected mutations" will develop as quite normal plants. Consequently the mutability of *O. pratincola* is probably not as great as might be assumed from the tables.

Mut. Mummularia a discontinuous variation

Critics of DE VRIES' work on mutation in *Oenothera Lamarckiana* have not infrequently expressed skepticism as to whether or not the

mutations were actually unconnected with the parent form by intermediates, which might have been overlooked in classifying the young seedlings. An endeavor has been made to forestall the same criticism of the writer's work on *O. pratincola* by the publication

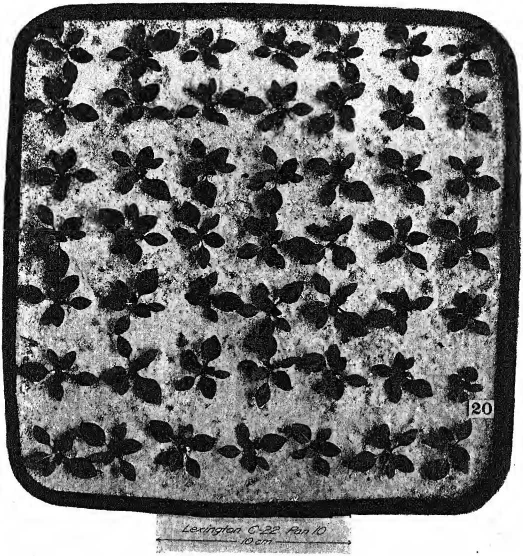

FIG. 12.—F_2 progeny of Lexington C, *Oenothera pratincola* (pan 10 of the progeny of C-22); the only mutation shown is C-22-20, mut. *nummularia;* the other plants are typical *O. pratincola.*

of a series of photographs showing some of the pans in which the mutations occurred. Each reader can judge for himself as to the discontinuity of the mutations from the rest of the plants. It is believed that no one has heretofore published so extensive a series of photographs representing random samples of cultures from which

none of the seedlings had been discarded. It must be remembered that each pan is a fair sample of a whole culture, for the seedlings were pricked off when very small and were taken from the seed pan as they came, with no attempt at sorting.

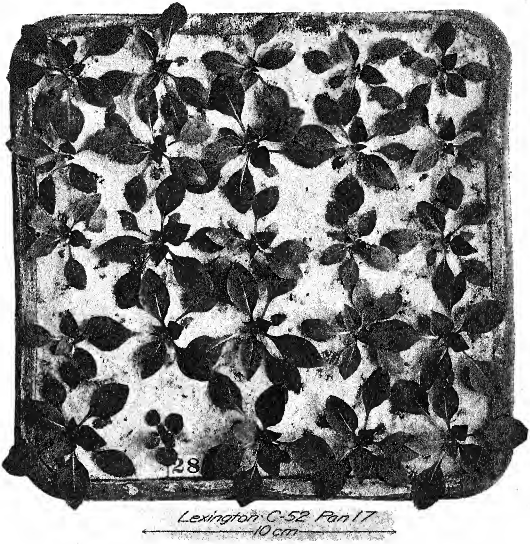

FIG. 13.—F_2 progeny of Lexington C, *Oenothera pratincola* (pan 17 of the progeny of C-52); one example of mut. *nummularia*, C-52–28, is shown; the remaining plants are typical.

Figs. 5, 6, 12, 13, 14, 15, and 17 show 7 of the 50 occurrences of mut. *nummularia* in cultures aggregating 13,035 plants. Three more of the original plants of this mutation are shown in figs. 3, 4, and 16. The figures showing entire pans should give a fairly clear idea of what the writer interpreted as fluctuating variation. It is believed that very few if any mutations escaped detection in the

cultures of 1914. The two mutations of Lexington C (nos. 28 and 57) which passed muster as typical plants when the seedlings of 1913 were examined would probably not have been missed in the

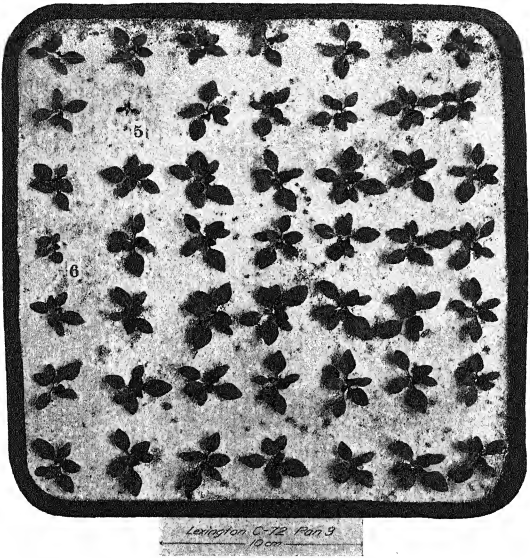

FIG. 14.—F_2 progeny of Lexington C, *Oenothera pratincola* (pan 3 of the progeny of C-72); two mutations are shown, mut. *subulata*, C-72-5, and mut. *nummularia*, C-72-6; the other plants are typical.

more searching scrutiny which the seedlings of 1914 underwent.[22] Although some of the mutations cannot be distinguished in the young seedling stage with ease, it is believed that the likelihood

[22] Mut. *nitida*, represented by Lexington C-57 in the cultures of 1913, occurred several times in 1914 and was detected in the young seedling stage in every case Nov. (1914).

of mistaking mut. *nummularia* for the parent type or for one of the other mutations is negligible. The orbicular seedling leaves are too striking a characteristic to be overlooked.

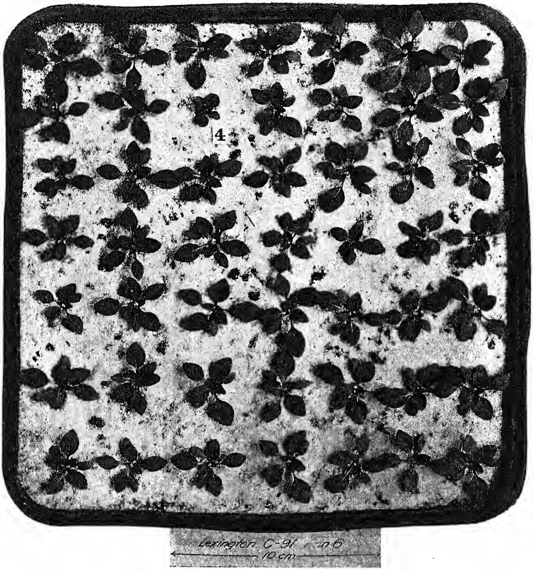

FIG. 15.—F_2 progeny of Lexington C, *Oenothera pratincola* (pan 6 of the progeny of C-91); one plant of mut. *nummularia* is shown, C-91–4; the other plants are typical.

The unlikeness of mut. nummularia and O. pratincola × O. numismatica

Before mut. *nummularia* had been found in F_2 progenies from guarded seed, it seemed possible that it might be an F_1 hybrid of *O. pratincola* with some other wild species, of which a few pollen

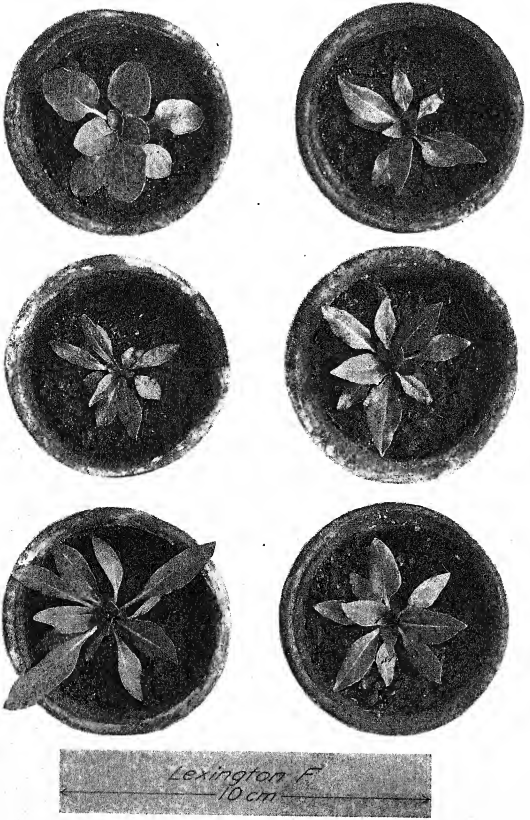

FIG. 16.—Mutations from the F_1 progeny of Lexington F, *Oenothera pratincola;* the plants are F-1, mut. *nummularia;* F-2, -4, -5, -6, mut. *rubricentra;* F-3, mut. *tortuosa* (?), taking the plants in order from the upper left-hand corner.

grains had accidentally reached the stigmas of the mother plants. This hypothesis was tested by crossing *O. pratincola* with *O. numismatica*. As already stated, these two species were the only Onagras which the writer found at Lexington. The latter, furthermore, is

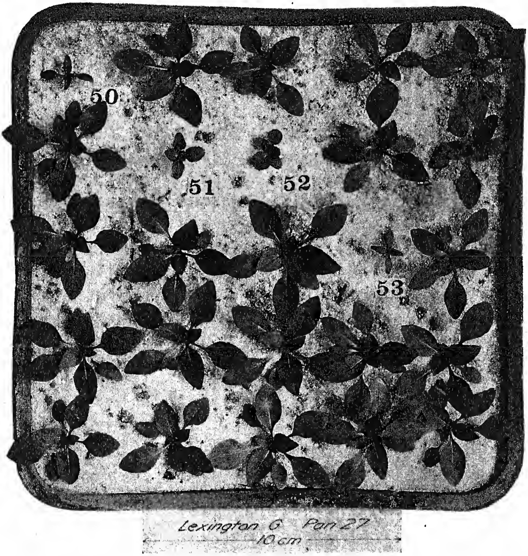

FIG. 17.—F_1 progeny of Lexington G, pan 27, *Oenothera pratincola;* four mutations are shown, G-50 and G-53, mut. *rubricentra;* G-51, mut. *nitida;* G-52, mut. *nummularia;* the other plants are typical *O. pratincola.*

suspiciously similar in several characters to mut. *nummularia*, as may be seen by comparing the characters already recorded. The cross *O. pratincola* ♀ × *O. numismatica* ♂ was conspicuously fertile; 326 seeds from one capsule gave a culture of 222 plants, consisting

of twin hybrids and one mutation. The solitary exception was broader leaved than the rest of the culture and is interpreted by the writer as the product of a cross between a mutated ♀ gamete, which if pollinated by *O. pratincola* would have yielded mut. *nummularia*, and a normal ♂ gamete of *O. numismatica*. This hypothesis will be tested later by appropriate crosses. It is clear that a cross between *O. pratincola* and *O numismatica* does not yield mut. *nummularia*, or anything resembling it, with greater frequency than does unhybridized *O. pratincola*. Perhaps *O. numismatica* is itself a mutation from *O. pratincola*, or a form which has segregated from the cross mut. *nummularia* × *O. pratincola*. One would expect the latter cross to occur rather often if, as seems to be the case, mut. *nummularia* itself is partially self-sterile. It seems not unlikely that in nature self-sterile or nearly self-sterile mutations may be perpetuated by effective cross-pollination, either as stable hybrids or as homozygous forms resulting from subsequent segregation. It is an interesting fact that although *O. pratincola* has a very high proportion of good pollen grains (90 per cent or more), mut. *nummularia* rarely has pollen which is 50 per cent perfect, and some anthers produce no good pollen at all.

Conclusions

1. *Oenothera pratincola*, a recently described small-flowered self-pollinating species from Kentucky, is in a mutating condition comparable with that of *O. Lamarckiana*.

2. The most striking of the mutations, *O. pratincola* mut. *nummularia*, occurred in strains derived from 7 wild mother plants out of 8 selected at random.

3. In two of these strains the mutation was found in both the F_1 and F_2 generations from the parent plant. In a third strain the mutation was found only in the F_2 generation, but a sufficient number of F_1 plants had not been grown to insure its detection in that generation.

4. Mut. *nummularia* appears to occur with a frequency of about one individual to each 300–400 seeds planted. The several progenies showed no significant variation in the mutation ratio.

5. The mutation ratio cannot be explained on Mendelian grounds.

6. Mut. *nummularia* is better adapted than the parent type to withstand influences unfavorable to germination. In every case where a progeny contained an unexpectedly large number of mutations, the germination was correspondingly poor.

7. Selective germination and differential mortality among dormant seeds may be important factors in natural selection.

8. Mutation is a distinct process from Mendelian segregation, and the phenomena exhibited by *Oenothera Lamarckiana*, *O. biennis*, and *O. pratincola* cannot be attributed to heterozygosis.

Bureau of Plant Industry
Washington, D.C.

THE EMBRYO SAC AND EMBRYO OF STRIGA LUTEA

MARGARET R. MICHELL

(WITH PLATES VIII AND IX)

Attention lately has been drawn to *Striga lutea*, a semi-parasitic plant belonging to the Rhinanthoideae-Gerardieae (8) group of the Scrophulariaceae, owing to the ravages caused by it in the maize crops in parts of South Africa.

The material for this investigation was obtained by Dr. H. H. W. PEARSON in Pretoria during the autumn of the years 1912 and 1913.

The ovaries were fixed in a chromacetic solution and the chief stain used was Haidenhain's iron-hematoxylin. A combination of diamant fuchsin and light green also gave good results, and Flemming's triple stain gave excellent differentiation in the embryonic stages.

Ovule and embryo sac

There is nothing striking in the ovary. It is of the ordinary bilocular scrophulariaceous type, and bears a large number of minute anatropous ovules on the rather swollen placentae. Many of the ovules possess long funicles, and in some cases the funicle branches and bears two ovules. The fact that the length of funicle varies enables the plant to produce a greater number of ovules per unit area of placenta than it would be able to do were the funicles all of one length.

The archesporium can be distinguished at an early stage before the integument arises. It consists of a single hypodermal cell which, without undergoing division, becomes directly the megaspore mother cell. This is shown in fig. 1, which also shows the origin of the integument. The young ovule grows with great rapidity, and before the first division of the nucleus of the megaspore mother cell, the integument is well marked and the whole ovule is rapidly assuming its mature anatropous form.

The nucellus consists of one layer of cells. As development proceeds the cells become flattened and finally disorganized, so that

when the embryo sac has reached maturity, only traces of it are to be found lying between the integument and the embryo sac.

The bulk of the ovule is composed of the thick integument, as is often the case in the Scrophulariaceae. Fig. 2 represents a transverse section through the ovule just above the integument and shows the nucleus of the mother cell in synapsis, prior to its first division. In the ovule drawn the nucleolus is just visible, but in many similar ovules it has disappeared. It was observed that, when synapsis occurs, about 10 per cent of the ovules in an ovary are in this stage simultaneously. Other stages in the heterotypic division were not seen, the next stage being that of the homotypic division (fig. 3). Fig. 4 shows the three upper megaspores degenerating, while the fourth has become the embryo sac.

The development of the embryo sac is perfectly normal. The nucleus divides and the resulting nuclei pass one to each pole of the embryo sac, the center being occupied by a large vacuole (fig. 5). These nuclei divide twice (figs. 6–8), thus giving two groups of four nuclei, one at each end of the sac. One nucleus from each group then moves toward the center (fig. 9); these two nuclei meet and fuse in the upper part of the sac, not far from the egg cell (fig. 10). BALICKA-IWANOSKA (1) found in certain genera of the Scrophulariaceae that the polar nuclei fuse about the middle of the embryo sac and migrate toward the egg at the time of fertilization. SCHMID (13) pointed out that this position of the polar nuclei at the time of fusion is not that always found in this family. He found that the position at the time of fusion may vary in a single species. For instance, in *Pedicularis palustris* the polar nuclei may fuse in the upper, lower, or middle part of the sac. In the cases in which fusion occurs in the middle or at the base of the sac, they migrate toward the egg at the time of fertilization.

The synergids have assumed a caplike appearance by the time the embryo sac is ready for fertilization. In the embryo sac shown in fig. 11 this cap is not yet developed, but the synergids are early distinguished from the egg by their much smaller size. In this figure the antipodals show signs of disintegration, and at the stage represented in fig. 12 a small deeply staining mass is all that is left of them.

In general behavior this embryo sac differs in no way from the rest of the Scrophulariaceae, in which it is a general rule that the synergids are well marked off from the other nuclei of the embryo sac, and the antipodals are inconspicuous, disappearing about the time of maturity.

Case of an ovule with two embryo sacs

In an ovary in which most of the ovules were in the 4-nucleate or 8-nucleate stages, one ovule was seen showing two sacs lying side by side. Fig. 18 represents a section through this ovule not passing through the embryo sacs. In this section the ovule has every appearance of having been derived from two fused ovules. Figs. 16 and 17 support this view, as the cells separating the two embryo sacs clearly belong to the integument. Unfortunately the sections through this ovule are oblique. Figs. 13–17 are drawn from this ovule as it was represented in five consecutive sections. The left-hand embryo sac has four nuclei arranged in two groups of two each. The right-hand embryo sac contains eight nuclei apparently not definitely arranged in groups. Six of the nuclei lie fairly close together at one end of the sac and two at the other end. Possibly this lack of arrangement is to be correlated with the abnormal conditions under which the sac has developed. This ovule was the only one showing any abnormal tendencies.

Cases in which two ovules have grown together to form one with two embryo sacs have not been reported as frequently as those in which the two embryo sacs arise from a single archesporium. They have been recorded, however, in *Pirus Malus*, *Loranthus europaeus*, and *Viscum album* (5).

Fertilization

Ovules in which fertilization is taking place are fairly abundant. In the section represented in fig. 12 the pollen tube is seen having penetrated the embryo sac and apparently pushed its way through one of the synergids, and has discharged its contents into the sac. One male nucleus is seen fusing with the egg, while the other is fusing with the nucleus produced by the fusion of the polar nuclei. The male nuclei may be easily distinguished from the nuclei of the

embryo sac by their much smaller size. This case is yet another to be added to the ever-growing list of plants in which double fertilization is known to occur.

Endosperm formation

The endosperm is initiated by cell formation. *Striga lutea* thus conforms with the type largely represented in the Sympetalae and almost universal in parasites and saprophytes (5).

The first division of the primary endosperm nucleus is immediately followed by the formation of a transverse wall, dividing the embyro sac into two chambers (fig. 19). The nucleus of the chalazal chamber divides once, but no wall is formed, and endosperm is never produced in this chamber. Its function is clearly haustorial, for soon after the division of the nucleus the chalazal end of the sac grows down into the integument, and finally curving upward reaches the outermost layer of the integument (fig. 22). Fig. 21 shows a somewhat earlier stage in which the remains of the antipodals are seen at the end of the haustorium.

The nucleus of the micropylar chamber divides three or four times, each division being accompanied by a wall transverse to the long axis of the sac (fig. 20). After this, walls appear in various planes and the original transverse walls are soon obscured. No definite micropylar haustorium is formed, though the cells of the endosperm grow a short distance up the micropyle, surrounding the suspensor, and are probably to be considered as having a haustorial function (fig. 23).

The endosperm cells around the suspensor (fig. 23) and those formed at the base of the original micropylar chamber (fig. 22) have dense protoplasm and stain far more deeply than the rest of the endosperm cells. This may be due to the fact that they are active in passing the food, which is gradually being absorbed from the integument by the haustoria, to the developing embryo. In these ovules there is no distinct tapetal layer round the embryo sac.

In the endosperm formation and in the development of haustoria there is a remarkably close resemblance between *Striga lutea* and *Linaria alpina*. SCHMID (13) in his account of *Linaria alpina* might well be describing *Striga lutea*, the chief difference being that

in *Striga* the chalazal haustorium seems to be slightly longer and to have a definite upward curve.

Embryo

After fertilization the egg cell does not divide immediately. Figs. 19 and 20 show the fertilized egg in the resting stage while endosperm is being formed rapidly.

The first division of the egg is transverse, the lower cell giving rise to the embryo, the upper to the suspensor, which develops rapidly and is divided into three or four cells by transverse walls (fig. 23). The cell of the suspensor nearest the micropyle increases in length far more rapidly than the others and crushes all the endosperm cells at its apex, thus coming to lie in contact with the integument. In appearance the proembryo is rather like that of *Physostegia* (14), and also, though shorter, bears a resemblance to that of *Myoporum serratum* (3). The cross-walls which are present in the suspensor of *Striga* and *Physostegia* are absent in *Myoporum*. A difference which becomes marked later in the development of the proembryo is the appearance of haustoria in *Striga*. These are chiefly confined to the basal cell, though in one case the cell below has produced a small lateral haustorium (fig. 26). LLOYD (10) in his account of the Rubiaceae shows that in many members of that family haustoria are developed from the suspensor, but as far as the writer has been able to ascertain, this has not been recorded for the Scrophulariaceae.

The embryonic haustoria are tuberous in form and do not show the slightest resemblance to those of the endosperm.

The first wall of the embryo proper is formed in a longitudinal plane (fig. 24) and is followed immediately by another longitudinal wall at right angles to the first, dividing the embryo into four cells (fig. 25). A transverse wall is then formed, dividing the embryo into octants. The next walls are periclinal (fig. 26).

Walls then follow in quick succession, giving rise to a spherical embryo (fig. 27) on the end of a long suspensor. The mature embryo is of the ordinary dicotyledonous type (fig. 28) and is surrounded by one row of endosperm cells.

Changes in endosperm and testa

At a very early stage in the formation of the endosperm, the outer wall of the outermost layer of endosperm cells becomes cutinized, and it is clearly impossible that food can pass through to the embryo, which must therefore derive its nourishment through the haustoria.

As the embryo grows, absorbing the food stored in the endosperm and integument, the walls of the outer layer of the endosperm become thickened, but are still composed of cellulose. It is these thickened cells which compose the endosperm in the mature seed. Proteid reserves alone are found, neither starch nor oil being present.

There is no well defined tapetal layer around the endosperm as is the case in most Scrophulariaceae already investigated.

All the food stored in the integument is absorbed by the haustoria and only the outermost layer of the integument persists in the mature seed. This layer undergoes a good deal of change. Its radial walls increase in size, assume a wavy outline, and become lignified. It is to this last fact that the brittleness of the testa is due.

It is a well known fact among farmers that the seed of *Striga lutea* may retain its capacity for germination for several years (11), owing no doubt to the protection afforded by the lignified testa and cutinized outer wall of the endosperm, as well as to the reserves of proteid material in the endosperm. When to this is added the fact that each ovary produces hundreds of seeds, practically every one of which is fertile, it is not difficult to realize why it is that this plant is so difficult to eradicate once it has obtained a hold on a crop.

Discussion

Since the middle of the last century the ovules of the Scrophulariaceae have from time to time been the object of investigation. Among the earlier workers on this subject are to be found DEECKE (6), TULASNE (15), HOFMEISTER (9), CHATIN (4), and others.

It is rather interesting to note that *Pedicularis sylvatica* was in 1855 the subject of a violent controversy between DEECKE and SCHACHT on one hand, and HOFMEISTER, VON MOHL, and TULASNE,

on the other. In 1835 SCHLEIDEN had expounded the theory that the pollen tube entered the embryo sac and there gave rise to the embryo, basing his conclusions on observations made on ovules of plants belonging to various families (5). In 1855 DEECKE (6) in *Pedicularis sylvatica* claimed to have seen the pollen tube entering the sac and developing there into the embryo, and came to the conclusion that in this plant he had proved beyond all doubt that SCHLEIDEN'S view as to the origin of the embryo was correct. SCHACHT (12) confirmed DEECKE'S statement, but HOFMEISTER (9) proved that what DEECKE had seen and drawn was the proembryo. He believed those figures in which DEECKE depicted the "pollen tube" wandering outside the micropyle to be due to roughness in dissection.

It is extremely interesting to find that the "pollen tube" of DEECKE bears a striking resemblance to the proembryo of *Striga lutea*. That it is a proembryo is obvious, since DEECKE shows it imbedded in the endosperm, and he also figures very clearly structures which in the light of SCHMID'S work on *Pedicularis* we may interpret as two lobes of a micropylar haustorium and a chalazal one. *Striga lutea* possesses a long suspensor which is clearly comparable with DEECKE'S pollen tube. In the majority of his figures the end of the "pollen tube" remote from the embryo forms a swelling much resembling that shown in the young proembryo of *Striga* (fig. 23). In DEECKE'S fig. 16 a case is shown of two "pollen tubes" entering one ovule. One of these tubes is traced down to the embryo, the other advances only a short way down the micropyle. The explanation of this phenomenon might be that in *Pedicularis sylvatica*, as in *Striga*, the basal cell of the suspensor produces haustoria and the second "pollen tube" is simply a haustorium. Of recent years the chief contributions to our knowledge of the embryo sac of the Scrophulariaceae have been made by BALICKA-IWANOWSKA (1) in 1899 and SCHMID (13) in 1906.

BALICKA-IWANOWSKA'S work is of a more general character than SCHMID'S, dealing with several sympetalous families, while SCHMID confines himself to a number of species selected from genera representing the three main groups of the Scrophulariaceae: Pseudo-

solaneae, Antirrhineae, and Rhinanthoideae. It is on this last group that most work has been done, though its subdivision Rhinanthoideae-Gerardieae, to which *Striga* belongs, has up to the present not been investigated. For this reason it is of great interest to discover how far *Striga* may be compared with the plants belonging to the Rhinanthoideae-Digitaleae and Rhinanthoideae-Rhinantheae. In all the plants which have been studied the features of the greatest interest are found in the post-fertilization phases of development and in this respect *Striga lutea* is no exception.

"Double fertilization" has been demonstrated in *Linaria vulgaris*, *Digitalis purpurea*, *Pedicularis foliosa*, and *Melampyrum silvaticum*, to which may now be added *Striga lutea*.

The development of haustoria and the development of endosperm go hand in hand and therefore may be treated together. Schmid (13) puts the genera he has studied into four groups according to their method of endosperm formation.

In the first he puts *Verbascum*, *Scrophularia*, and *Digitalis*. In these genera four superposed primary endosperm cells are formed, of which only the two inner ones form the true endosperm, the uppermost and lowermost cells dividing into four and assuming a haustorial function, though in a much less marked degree here than is found in the other groups.

Linaria and *Antirrhinum* constitute a second group. In them a transverse division of the embryo sac occurs. The upper cell gives rise to the endosperm, of which a few cells at the micropylar end function as a haustorium; the lower cell grows out into a tube-like haustorium, in which no cell walls are formed though the original nucleus divides once.

The third group contains *Alectorolophus* (*Rhinanthus*) and part of the genus *Lathraea*, which Schmid includes in the Scrophulariaceae. Here, as in the second group, the lower cell becomes a haustorium without septation, and two cells arising at the micropylar end of the upper cell give rise to a micropylar haustorium. Each of these cells has two nuclei.

Veronica, *Euphrasia*, *Pedicularis*, *Melampyrum*, and *Tozzia* are put in the fourth group, which differs from the third only in that

the micropylar haustorium arises from a single cell and contains four nuclei.

SCHMID has made it clear that the different species of a genus closely resemble one another in the character of the endosperm and haustoria, and he believes that these characters should receive consideration in drawing up a natural classification of the family. Judging things from this point of view, the Rhinantheae appear to be a natural group, since all the members studied show an extraordinary similarity of development. It certainly is striking that *Linaria* and *Striga*, which are widely separated in the present system of classification, should show such close agreement in their development of endosperm and haustoria. Though SCHMID has not paid much attention to the proembryo, it seems that here also is a strong resemblance. Surely there must be a close relationship between genera which show agreement in minute details of the development of organs which do not seem to be influenced by environment to nearly the same extent as those organs which are most used in drawing up a scheme of classification.

DOP (7) finds in *Buddleia* a remarkably close resemblance to *Verbascum*, *Scrophularia*, and *Digitalis* as regards the mode of formation of the endosperm and haustoria. He concludes that the evidence obtained emphasizes the relationship between the Scrophulariaceae and Buddleiaceae (if the latter be regarded as a family distinct from the Loganiaceae as WETTSTEIN [16] thinks it ought to be), and that it tends to separate the Buddleiaceae more widely from the Solanaceae.

The question whether parasitism of the plant as a whole affects the embryo sac and embryo has often been raised. The evidence afforded by the Scrophulariaceae all tends to show that these structures are not influenced by the habit of the plant. Extensive development of the haustoria is certainly characteristic of the Rhinantheae, but *Striga*, which is also a semi-parasite, is not so characterized, while *Veronica*, a non-parasite, has practically the same degree of haustorial development as the Rhinantheae. It seems, therefore, that intensity of haustorial development is not to be correlated with the parasitic habit of the plant.

BERNARD (2) was led to the same conclusion from a study of four total parasites: *Lathraea*, *Orobanche*, *Phelipaea*, and *Cytinus*, in which he found that all stages of haustorial development were represented. *Lathraea* shows the most extensive haustorial development of the four genera, while in *Cytinus* the endosperm is normal or nearly so.

In the production of fertile seeds the parasitic members of the family seem not one degree less successful than their independent relatives, and there is nothing in the history of the ovule of parasites to lead one to suppose that it has suffered owing to the mode of life adopted by the parent plant.

Summary

1. *Striga lutea* is a semi-parasitic annual belonging to the Rhinantheae-Gerardieae group of the Scrophulariaceae.

2. The ovary is of the ordinary bilocular scrophulariaceous type, and the ovules are anatropous, with one integument.

3. The megaspore mother cell arises directly from a single subepidermal cell, which gives rise to a row of four megaspores, of which the lowest develops into the embryo sac.

4. The 8-nucleate embryo sac develops in the normal way, and at the time of fertilization contains two synergids, an egg, the fused polar nuclei, which lie in the upper part of the sac, and three antipodal cells, which show signs of disintegrating.

5. Double fertilization occurs.

6. Endosperm is formed by cell division. From the chalazal end a long binucleate haustorium is formed, penetrating the integument. The micropylar haustorium is inconspicuous, simply consisting of a few ordinary endosperm cells with fairly dense contents.

7. The proembryo has a long suspensor of three or four cells. The basal cell of the suspensor forms tuberous haustoria.

8. The mature embryo is of the usual dicotyledonous type and is surrounded by one thick-walled layer of endosperm cells.

9. The testa consists of one layer of lignified cells which are admirably suited to protect the young embryo.

In conclusion I should like to take this opportunity for thanking Dr. H. H. W. PEARSON for his help during the early stages of this work, which was started at the South African College, and for his continued interest in its progress. I am indebted to Professor SEWARD for kind permission to work at the Cambridge Botany School, and to Mr. R. P. GREGORY for many helpful criticisms.

THE BOTANY SCHOOL
CAMBRIDGE, ENGLAND

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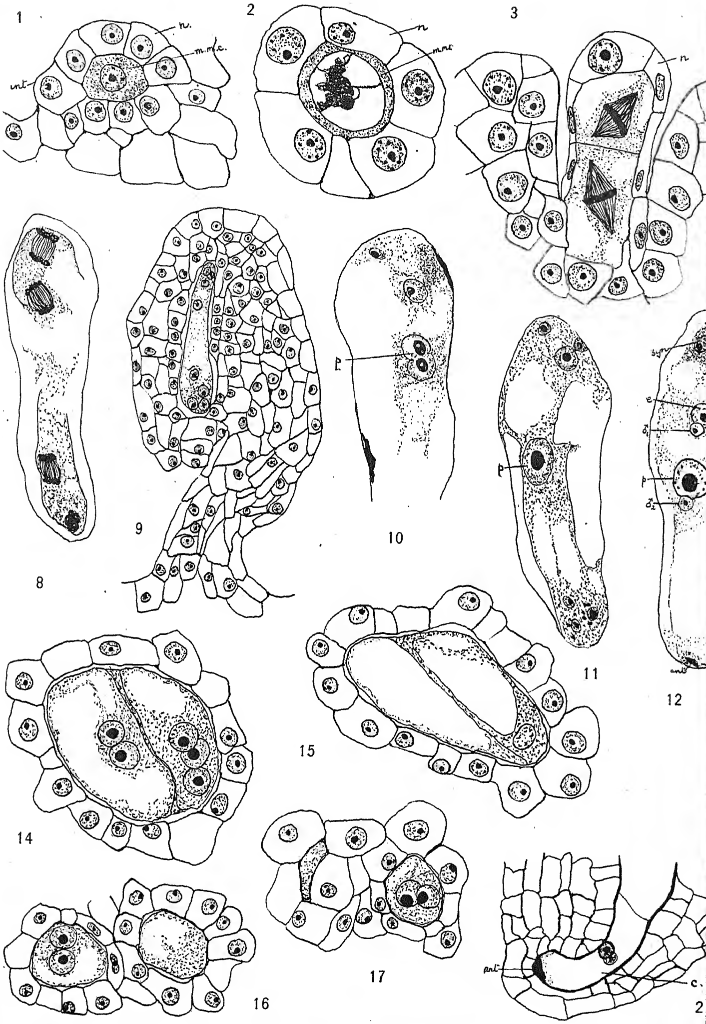

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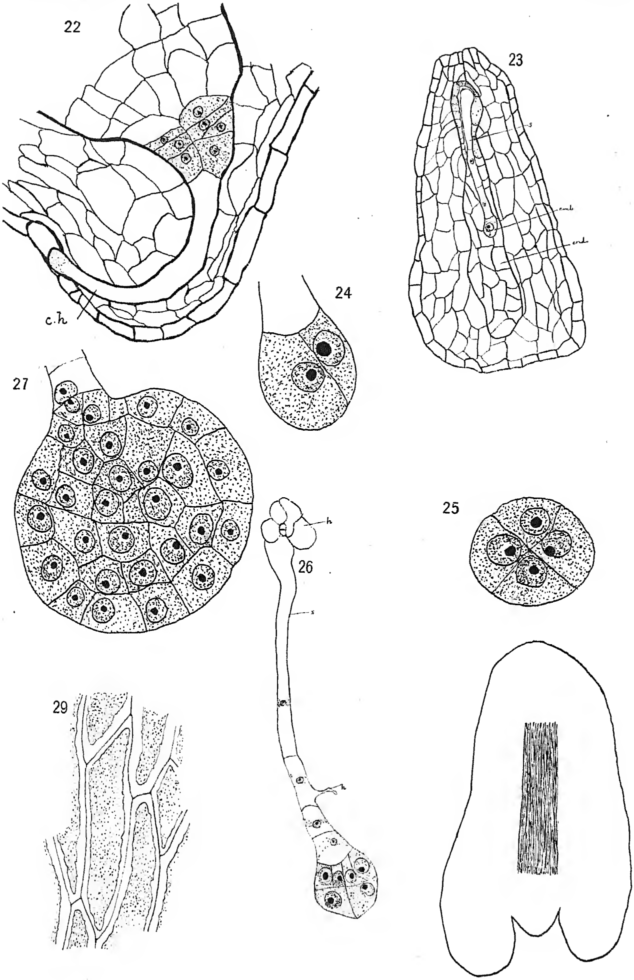

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EXPLANATION OF PLATES VIII AND IX

Abbreviations: *mmc*, megaspore mother cells; *int*, integument; *n*, nucellus; *dm*, degenerating megaspores; *p*, polars; *syn*, synergids; *ant*, antipodals; *e*, egg; *pt*, pollen tube; *o*, oospore; *ch*, chalazal haustorium; *emb*, embryo; *end*, endosperm; *s*, suspensor; *h*, haustorium.

PLATE VIII

FIG. 1.—Young ovule showing the megaspore mother cell and the origin of the integument; ×1210.

FIG. 2.—Megaspore mother cell with the nucleus in synapsis; ×1470.

FIG. 3.—Homotypic division of the megaspore mother cell nucleus; ×1470.

FIG. 4.—Ovule showing the embryo sac and three degenerate megaspores; ×1150.

FIG. 5.—Binucleate embryo sac; ×1210.

FIG. 6.—Division of the two nuclei; ×1210.

FIG. 7.—Tetranucleate sac; ×1210.

FIG. 8.—Division of the four nuclei; ×1210.

FIG. 9.—Ovule showing an 8-nucleate sac; ×1210.

FIG. 10.—Polar nuclei fusing; ×1210.

FIG. 11.—Mature embryo sac, just before fertilization; ×1210.

FIG. 12.—Fertilization; ×1210.

FIGS. 13–17.—Abnormal ovule with two embryo sacs; ×1210.

FIG. 18.—Section through this ovule not showing the embryo; ×520.

FIG. 19.—After the first divisions of the primary endosperm nucleus; ×1210.

FIG. 20.—Later stage in endosperm formation; ×1210.

FIG. 21.—Part of an ovule showing the young chalazal haustorium; ×520.

PLATE IX

FIG. 22.—Older chalazal haustorium; ×520.

FIG. 23.—Proembryo imbedded in endosperm; the chalazal haustorium cannot be seen in this section; ×260.

FIGS. 24–27.—Stages in the development of the embryo; figs. 24, 25, 27, ×1210; fig. 26, ×575.

FIG. 28.—Embryo from the mature seed; ×71.

FIG. 29.—Surface view of the testa of the ripe seed; ×605.

THE ORIGIN OF THE INFLORESCENCES OF XANTHIUM

CLIFFORD H. FARR

(WITH PLATE X)

The bur of *Xanthium*, together with the two inclosed seeds, has been the subject of considerable investigation for many years. ARTHUR (1) in 1895 confirmed the popular notion that the germination of one of the seeds is delayed approximately a year beyond the other, and also that it is the lower and better developed seed which germinates first. CROCKER's experiments (5) in 1906 indicated that this phenomenon is due to a difference in the permeability of the seed coats to oxygen. In 1911 SHULL (14) demonstrated further that the embryos differ in the amount of oxygen required for germination; and the same writer (15) has published an article more recently on the nature of the semipermeability of the seed coats. In addition to these physiological studies, the structure of the seed coats has been investigated by HANAUSEK (10).

The morphology of the pistillate inflorescence, or bur, has been much discussed and variously interpreted. The oldest and most generally accepted view is that which considers it a fusion of involucral bracts. This was supported by WARMING (16) and ROSTOWZEW (13), and accepted by ARTHUR, GRAY, and BRITTON. As early as 1838 BRASSAI (3) dissented from this interpretation and referred to the bur as a fusion of many floral bracts. Later BAILLON (2) modified BRASSAI's idea by assuming a union of but two floral bracts, coalesced along their margins; and GOEBEL in his most recent paper (9) accepts this explanation. However, only three years before, the last named writer (8) presented another possibility, namely, an intercalary growth about the base of the flowers, enveloping them and carrying their floral bracts upward to form the beaks.

There is also a marked difference of opinion as to the morphology of the spines which are so prominent on the mature bur. CLOS (4) suggested that these are new structures ("Emergenzen"),

and he has been followed in this by many workers, including BAILLON (2) and GOEBEL (9). However, KOEHNE (12) as long ago as 1869 conceived of them as involucral bracts; while HOFMANN (6) referred to them as "Spreublätter"; and ROSTOWZEW (13) simply called them modified bracts.

These diversities of interpretation have doubtless been due to an inadequate knowledge of organogeny, and to an incomplete correlation of the spines, bracts, etc., of related genera. A considerable number of typical Compositae have been investigated and the morphology of their parts is well established. The Ambrosiaceae, the tribe to which *Xanthium* belongs, has been excluded from the Compositae by some, though several of its genera approach very near to the typical form of Compositae. *Iva* is without doubt that member of the Ambrosiaceae most closely resembling these Compositae, and a recent discussion of *Iva xanthiifolia* Nutt. by the writer (7) involved the homology of its bracts and rudimentary structures with corresponding organs in the inflorescence of the typical Compositae. This paper also presented a hypothesis as to the origin of dicliny in that species. The present investigation was undertaken in the hope that the results of the preceding study might aid in the explanation of the dicliny in *Xanthium* and the interpretation of its peculiar pistillate inflorescence. I wish to express my appreciation for the encouragement and suggestions of Dr. R. B. WYLIE under whose supervision the work was pursued; and thanks are also due to Dr. J. C. ARTHUR for assistance in the determination of species.

The staminate inflorescence

The species investigated was *Xanthium commune* Britton, in which staminate and pistillate capitula are associated on the same branch. The staminate head, which bears 150–175 flowers, is often solitary and always terminal, thus occupying an exposed position. The peduncle (fig. 2) on which it rises above the pistillate heads is quite slender in comparison with similar structures. Its length nearly always equals or exceeds the diameter of the head which it bears; while its own diameter is only about one-sixth as great. Three vascular bundles (fig. 4) run throughout the length of the peduncle,

and the vestiges of three others appear in its lower portion (fig. 3). These vestiges suggest that the peduncle of the staminate head once possessed a more extensive vascular system. This conclusion is further supported by the larger number of bundles in the base of the pistillate head. GOEBEL (9), in explaining the existence of the two or three bundles in the peduncle of the staminate head of *Ambrosia*, made use of a similar hypothesis of reduction, though he presented neither of the above lines of evidence. In *Xanthium* we find that now only three bundles conduct all the water for 150 or more staminate flowers. This supply seems all the more meager when it is noted how scantily this head is protected against water loss. Only a few involucral bracts mature, and these are so small as to be of scarcely any service to the capitulum in its older stages.

The conical receptacle (fig. 1), when young, so closely resembles a developing spike that WARMING'S generally accepted theory as to the spicate origin of the capitulum is at once recalled. As in most Compositae, the marginal flowers appear first, and the apical region maintains its meristematic nature for some time. Each flower is subtended by a cylindrical or spinelike floral bract which, though furnishing slight protection, probably does not appreciably lessen the amount of transpiration.

The parts of the flower appear in centripetal order. A lobed corolla is followed by a whorl of four or five stamens, and finally a pair of carpels. About one-third to one-half of the flowers in each head have only four stamens, not even the rudiment of a fifth appearing. In flowers with five stamens the corolla always possesses five equal lobes. In those with four stamens the corolla may have either four equal or five unequal lobes. In the last case two lobes frequently represent quadrants, while the other three make up the remainder of the cycle. Evidently the primitive staminate flower of *Xanthium* was pentamerous with respect to petals and stamens. It follows, therefore, that in some cases a reduction has taken place in the number of stamens and corolla lobes, the former yielding more readily than the latter.

The stamens are always united by their filaments. Though ROSTOWZEW (13) and JUEL (11) report that the anthers never fuse, in *Xanthium commune* the adjacent anthers are sometimes joined

in the same manner as in *Iva* (7). The fused cutinized layer is, however, not quite as thick as in the latter form. The similarity between *Xanthium* and *Iva* further extends to the ringlike enlargement at the base of the abortive style. These facts only serve to emphasize the kinship of these two genera with the main body of the Compositae.

ROSTOWZEW (13) stated that the stigma of the abortive style probably does not represent two carpels. In *Iva,* though this abortive stigma is not bifid at maturity, the appearance of a notch at the apex during development indicates derivation from the typical bifid form. Since no such notch appears in *Xanthium,* it seems probable that this genus has gone one step farther and obliterated even this last clue to the evolution of this structure. The close relationship of this form of *Iva,* however, strongly suggests that this rudimentary style arose from the usual bifid form.

The pistillate inflorescence

The fertile flower is axillary, being subtended (figs. 4–6) by a leaf or another pistillate head. Many instances of aborting leaves in such relation explain the frequent absence of subtending structures at maturity. The nearly sessile capitulum is attached to the floral axis by a large base which is over twice the diameter of the peduncle of the staminate head and contains 24 bundles (fig. 5). This vascular supply, in contrast to that of the staminate head, seems remarkably extensive when it is recalled that the pistillate head bears only two flowers, while the staminate has 150 or more.

The involucral bracts, 9–15 in number, appear first and arch over the young head, protecting it very effectively. The proximal ends of the adaxial bracts are closely crowded between the receptacle and the stem, and those on the abaxial side between the receptacle and the subtending structure. Meanwhile the tips of these recurved bracts (fig. 6) come in touch with the apex of the growing receptacle near its center. It seems that this contact temporarily arrests development in that region of the receptacle, for it immediately becomes flattened. Soon continued growth of the margin of the receptacle results in the formation of an apical depression (fig. 7). It may be, of course, that this sequence of

events has become fixed in the life-history, but arrest of development in the center of the receptacle due to contact with the apices of the involucral bracts might explain its origin both in individual and racial development. In the light of the foregoing it is evident that the terminal heads develop as in the normal Compositae, because the involucral bracts are not hemmed in by subtending structures and hence their tips probably do not come in contact with the receptacle.

Growth in the marginal region is lateral as well as longitudinal, making the depression larger at the bottom and constricted above. Furthermore, the floor of the pit is never a uniformly concave surface, but is slightly elevated at its exact center (fig. 7). This elevation soon resumes growth (fig. 8) and gives rise to a septum dividing the depression into two chambers (fig. 9). Later this septum develops in its upper surface a cleft which may extend downward some distance (fig. 13).

Many papillae early cover the marginal surface of the receptacle (figs. 8 and 9), as in the staminate head. Those of the latter, it will be recalled, give rise to the flowers and floral bracts, while these papillae of the pistillate head form hard hooked spines (fig. 13). That these spines are homologous with the floral bracts of the staminate head seems reasonably certain. It is hardly likely that flowers would become transformed into such structures by any process of evolution. These spines have the form and spiral arrangement of floral bracts. Furthermore, ROSTOWZEW (13) has shown that the vascular anatomy is like that of bracts. To consider them new structures would be to assume the disappearance of floral bracts and the subsequent appearance of structures similar to them in exactly the same location. Such a substitution is not supported by the observed facts. It seems, therefore, that there is abundant evidence for considering these structures floral bracts.

The margin of the head grows more rapidly on one side, causing one of the depressions to become deeper. This unequal growth also accounts for the peculiarity noted by ARTHUR (1) that the bur is flat on one side and curved on the other. The two flowers appear simultaneously, one at the bottom of each pit (fig. 8); but the lower, being in the deeper depression, is always larger, grows more rapidly, and at all stages of development is

more advanced than the upper. This difference persists even after fertilization, and in every case the lower seed bears the larger embryo. It is evident that the lower flower is in closer connection with the vascular supply, and possibly this circumstance, together with its priority in development, may have made a difference in the nutrition and water relations of the two flowers. These factors doubtless condition to a large extent the structure and composition of the seed coats and embryo, which in turn have been shown to influence germination. It thus appears that a difference in the rate of growth of the two sides of the young head and consequently the vertical displacement of one of the seeds results ultimately in a difference in the periods of delayed germination.

The sequence of development is the same for both flowers in the bur. The abortive corolla forms at first a complete ring (fig. 10), but before maturity it disappears entirely on the outer side (fig. 11). The two carpels, though appearing later, grow more rapidly than the corolla (fig. 10), and produce the bifid stigma which projects through the beaks at the time of pollination. One instance was noted in which two collar-like structures were present, the inner reaching but half-way around the base of the style. The outer, being a complete ring, is unquestionably the normal rudimentary corolla, and the inner should doubtless be considered the vestige of an abortive whorl of stamens, such as regularly appears in *Iva* (7).

A small rudiment of a floral bract is usually noticeable on the outer side of the base of the pistillate flower (fig. 12). It will be recalled that these flowers arise in deep cavities, the walls of which crowd closely about them on all sides. There is scarcely any space for the development of a floral bract therefore, and probably this crowding accounts for its reduction to the present dwarfed condition. Its presence, nevertheless, even though much reduced, precludes the possibility of considering the beaks to be the floral bracts of the subtended flowers.

Discussion

If the bur of *Xanthium* in its individual development follows at all closely the course of its evolution, there seems no doubt of its being a modified capitulum. The involucral bracts arise in the

usual way; the floral bracts all develop into spines except those of the two inclosed flowers, which remain rudimentary; and the "Anlagen" of the two flowers which appear follow the normal sequence of development. The only respects in which this head differs from those of the typical Compositae are seen in the two beaks and the depressions which they subtend. The beaks cannot be considered as floral bracts of the subtended flowers, because the rudiments of these parts are present in the pits. Neither is it likely that they are the floral bracts of aborted flowers, for they are quite unlike the spines in form, structure, and development. The fact that they bear the spinelike floral bracts on their outer surfaces precludes their being interpreted as modified involucral bracts. Moreover, two whorls of involucral bracts have never been established for any of the Ambrosiaceae. Though some writers (13) have interpreted *Iva* as having a double whorl of involucral bracts, my study (7) has shown that the inner of these whorls might properly be regarded as the floral bracts of the pistillate flowers. It seems most reasonable, therefore, to interpret the beaks of *Xanthium*, not as modified bracts nor even as newly developed structures, but as portions of the receptacle formed by its upward growth, and slightly altered by proximity to the depressions. Under this interpretation the only modification required to transform a typical head of Compositae into a *Xanthium*-like bur is the formation of two depressions in the apical region. In this species it seems that these depressions arose, not by a sinking of the flowers into the receptacle as HOFMANN (6) suggests, nor by an intercalary growth in the surrounding regions, but rather by an arrest of development in the apex due possibly to contact with the tips of the involucral bracts at an early stage.

In a recent treatise on sexual differentiation in plants (8) GOEBEL ascribes to modifications of nutrition, not only the origin and evolution of sex, but all the phenomena of hermaphroditism, dicliny, and dioecism. He surveys the entire plant kingdom and applies his interpretation to both cryptogams and phanerogams, and to gametophyte and to sporophyte alike. Numerous experiments and observations indicate that in many cases nutrition does determine the sex of an individual or part. But, especially in the

higher plants, it is by no means certain that this is the only conditioning factor, and in some instances it seems to influence sexuality only remotely, if at all. An illustration of this last condition is found in *Iva xanthiifolia* (7). In it the staminate flowers seem to have been evolved by an abortion of the pistils, probably on account of exposure to excessive transpiration. On the other hand, the pistillate flowers appear to have arisen by an abortion of the stamens, brought about evidently through pressure on the terminal portion of the flower and consequently lack of space in which to develop. That such transitions could take place is evidenced by the fact that, a priori, stamens are better fitted both in structure and in function to endure desiccation than are pistils; while carpels, owing to their central (and in an epigynous flower, basal) position, are less susceptible to pressure and crowding.

Xanthium, a form with pistillate and staminate flowers in different heads, affords even stronger evidence in favor of this interpretation. In many respects the staminate flowers are more exposed to factors accelerating transpiration than are the pistillate. The staminate heads are terminal, peduncled, and not subtended by protecting leaves. Each is composed of 150 or more flowers, and is supplied by only three vascular bundles. Moreover, the flowers are borne on a highly convex receptacle, and are protected by only a few relatively small involucral bracts, while their floral bracts are mere spines. Since all these conditions are in marked contrast with those under which the pistillate flowers develop, it seems reasonable to conclude that abortion of pistils in flowers of terminal heads was due to lack of a water supply adequate for the high rate of transpiration.

On the other hand, the pistillate flowers are obviously subjected to greater lateral pressure and have only a limited space in which to develop. They occur in pits which are constricted at their openings, and are enveloped by a dense spine-covered bur. Furthermore, there is a mutual reduction of all floral appendages, including corolla, stamens, and floral bract, indicating that this abortion is due to an external cause. It seems probable, therefore, that the arrest of development in the stamens, and hence the derivation of

the pistillate flower, is a direct consequence of crowded conditions during growth.

The differences between the staminate and pistillate flowers with respect to their protection, vascular supply, and number per inflorescence have been variously interpreted. They have been considered either as secondary sexual characters, or as a direct consequence of the difference in sexuality. In the former case the characters associated with the pistillate inflorescence would be thought of as having no essential relation to the nature or function of the female sex. In the second case it would be held that the pistillate flower is protected because it is in need of protection. It is apparent that my interpretation follows neither the incidental nor the teleological views above named, but ascribes to the characters associated with each kind of flower a rôle of primary importance *in effecting* the transition from hermaphroditism to dicliny. In other words, many of the differences between pistillate and staminate flowers appeared while the flowers were still perfect; and the effects of their presence occasioned the abortion of pistils and stamens respectively, resulting in the dicliny of this species.

Ambrosia and *Franseria* resemble *Xanthium* not only in their type of dicliny, but also in the peculiar burlike female inflorescence, the principal difference being that they have but one flower in each pistillate capitulum. Although there is need of further investigation, it may be well at this time to note what relation these genera bear to the conclusions reached above. ROSTOWZEW (13) stated that the pistillate heads of both forms are found in the axils of leaves. Furthermore, GOEBEL (9), in referring to *Ambrosia*, said, "In no instance have I observed in the male capitula even a trace of a subtending bract." The vascular supply has been studied only in the peduncle of the staminate head of *Ambrosia*, in which the last named writer found only two or three bundles. It is a matter of common knowledge that in both these genera staminate heads are terminal and peduncled, while pistillate are axillary and sessile. Evidently, therefore, *Ambrosia* and *Franseria* agree closely with the conditions in *Xanthium* as regards the position, protection, and, so far as known, the vascular supply of the heads. Thus it seems likely that the foregoing interpretation of the origin

of dicliny and of the peculiar fertile inflorescence may prove applicable to other genera of the Ambrosiaceae.

Summary and conclusions

1. The pistillate and staminate heads of *Xanthium commune* may be contrasted with respect to the following characters: position, attachment, subtending structures, number of involucral bracts, number of vascular bundles in the peduncle, number of flowers, and form of receptacle.

2. The pistillate and staminate flowers differ in degree of development of pistil, corolla, and floral bract. The stamens completely abort in the pistillate flower.

3. The vascular system in the peduncle of the staminate head has doubtless undergone reduction in the number of bundles.

4. The number of stamens per staminate flower is probably now undergoing reduction.

5. The anthers occasionally fuse, indicating relationship to the typical Compositae.

6. The bur is a modified capitulum, differing from the typical head of Compositae chiefly in the two depressions in the receptacle. These pits originate through a temporary arrest of development, which may possibly be attributed to contact with the tips of the recurved involucral bracts. This recurving of the bracts may be the result of limited space due to the subtending structures.

7. The spines of the bur are probably modified floral bracts.

8. The beaks seem to be modified portions of the receptacle.

9. The terminal heads became staminate, because the vascular supply was inadequate to compensate for the excessive transpiration, and hence the pistils have aborted.

10. The axillary heads became pistillate by the abortion of stamens, owing to the pressure and crowding incident to the formation of the flowers in depressions.

11. Many of the characters in which the pistillate and staminate flowers of *Xanthium* differ have been causative factors in the origin and development of dicliny in this form.

STATE UNIVERSITY OF IOWA
IOWA CITY

NOTE.—Since submitting the above for publication there has appeared an extensive study of abortive stamens by CURT SCHWARZE.[1] The author does not discuss the Compositae in this connection at all, nor does he refer to any recent work on that group. On the whole it presents very interesting corroborative evidence bearing on the conclusions reached above, when properly interpreted. Dr. SCHWARZE calls attention to SCHUMANN (1890) as the originator of the theory that reduction of stamens comes about through mutual external pressure of the organs in the bud. As opposed to this, he presents the contentions of FAMILLER (1896), MUTH, and others that abortion is due to internal factors, and himself suggests that these internal factors are constitutional in the protoplasm. This internal causation hypothesis is based on the failure in some cases to observe the parts in actual contact during development. It is evident that this is a very difficult point to demonstrate, as it necessarily involves disturbing the organs, which may itself separate surfaces loosely in contact. Moreover, recent investigations have served to greatly emphasize the delicacy of the sensitivity of plants to contact stimuli. But even granted that contact does not occur during the ontogeny of certain forms, there is still no reason that SCHUMANN and FAMILLER may not both have a correct interpretation. If we presume that the contact and mutual pressure did occur during the ontogeny of the ancestors of the living forms, such as is at present so striking in the Ambrosiaceae discussed above, it may be properly concluded with SCHUMANN that the pressure did cause an abortion. Such a reduction in size and arrest of development at an immature stage would necessarily involve a reduced vascular supply. Such a condition would doubtless result in diverted nutrition and water supply, which after many generations might make development of the stamens to maturity impossible even in the absence of mutual pressure. Thus by the reduced vascular supply becoming hereditarily fixed, there would be an internal cause for the abortion of the stamens, traceable to an original external condition. That the vascular supply is thus

[1] SCHWARZE, CURT, Vergleichende Entwicklungsgeschichtliche und histologische Untersuchungen reduzierter Staublatter. Jahrb. Wiss. Bot. 54:183–243. 1914.

modified is indicated by the observations of SCHWARZE, in that the cells of abortive stamens are often more vacuolate than those of stamens developing to maturity.

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EXPLANATION OF PLATE X

The figures were made with a camera lucida. The magnifications given are those of the drawings before being reduced one-half in reproduction. The abbreviations used are as follows: *b*, beaks; *s*, spines; *i*, involucral bracts; *f*, floral bracts; *a*, abortive corolla; *r*, rudimentary floral bract; *p*, pistillate head; *c*, carpels; *l*, leaf; *w*, pistillate flower.

FIG. 1.—Apex of floral axis, showing a staminate head with two pistillate heads appearing at its base; $\times 63$.

FIG. 2.—Developing staminate head; $\times 63$.

FIG. 3.—Transverse section of lower end of peduncle of staminate head; $\times 70$.

FIG. 4.—Transverse section of upper end of peduncle of staminate head; $\times 90$.

FIG. 5.—Young pistillate head, showing recurved bract; $\times 80$.

FIG. 6.—Young pistillate head, slightly older; $\times 80$.

FIG. 7.—Young pistillate head, showing developing depression; $\times 120$.

FIG. 8.—Young pistillate head, showing flowers appearing in the pits; $\times 85$.

FIG. 9.—Transverse section of pistillate head with flowers partly developed; $\times 90$.

FIG. 10.—Young pistillate flower, with carpels appearing; $\times 70$.

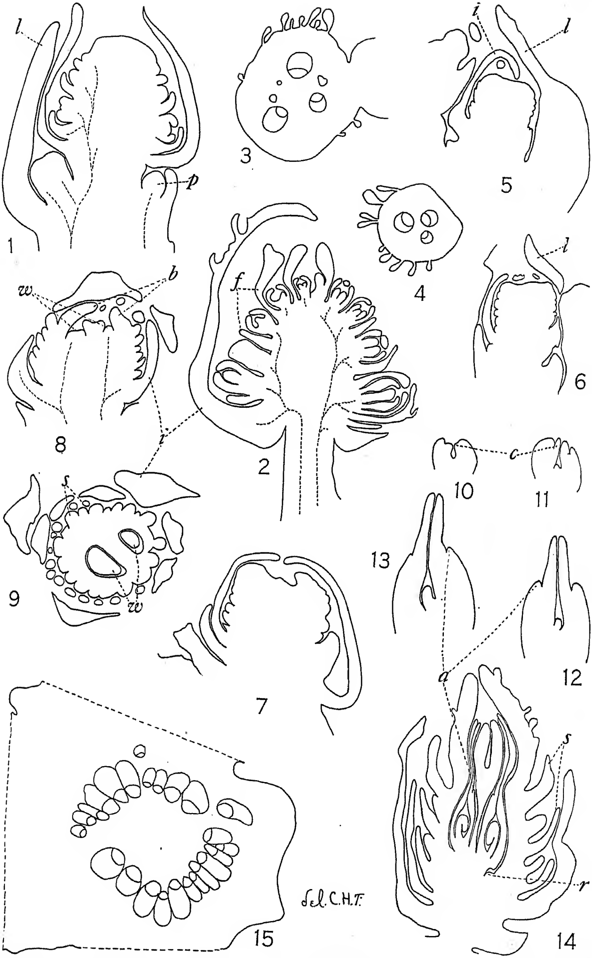
FIG. 11.—Young pistillate flower from lower pit of same head as in fig. 10; $\times 70$.

FIG. 12.—Young pistillate flower, showing ovule appearing; $\times 90$.

FIG. 13.—Young pistillate flower from lower pit of same head as in fig. 12; $\times 90$.

FIG. 14.—Pistillate head at megaspore mother cell stage; $\times 16$.

FIG. 15.—Transverse section of base of pistillate head, showing bundles; $\times 70$.



FARR on XANTHIUM

THE VISIBLE EFFECTS OF THE SCHUMANN RAYS ON PROTOPLASM[1]

W. T. BOVIE

Since the pioneer studies of DOWNES and BLUNT, a large number of investigators have studied the effects of ultra-violet light on protoplasm. Their investigations, however, have all been made on the effect of light of wave lengths longer than 2000 Angstrom units. This paper is a preliminary report of the visible effects produced in protoplasm by light waves shorter than 2000 Angstrom units.

When studying the biological effects of light, it is convenient to divide the spectrum into the following regions:

Regions	Wave lengths in Angstrom units
Infra-red	from 10,000 to 7,200
Visible	" 7,200 " 4,000
Ultra-violet of sunlight	" 4,000 " 2,950
Quartz ultra-violet	" 2,950 " 1,850
Fluorite ultra-violet	" 1,850 " 1,250
(Schumann region	" 2,000 " 1,250)
Lyman region	" 1,250 " 900

To this series we may add Röntgen rays with wave lengths from 1 to 0.1 Angstrom units, and gamma rays with still shorter wave lengths.

The effect on protoplasm of light of the Schumann region of the spectrum is particularly interesting because in this region of the spectrum the destructive action of the light is much more violent than it is in regions of longer wave lengths. This violent action is undoubtedly connected with the fact that the Schumann region of the spectrum is a region of general absorption for nearly all substances. Even substances as transparent as air and water absorb the shortest Schumann rays. A layer of air 1 cm. thick or a layer of water only 0.5 mm.[3] thick absorbs all except the longest Schumann rays. Fluorite, the most transparent substance

[1] Preliminary communication. [2] 10,000 Angstrom units = 1 micron.

[3] LYMAN, T., Astrophysical Jour. 27:87. 1908; Nature 84:71. 1910.

known, is the only solid which transmits the entire Schumann spectrum. It is interesting from a biological point of view to note that the absorption bands of such substances as egg-white and gelatin begin in the longer wave lengths before we reach the Schumann region.

In the writer's observations, to be described below, unicellular organisms were exposed to the Schumann rays and observed microscopically during and after the exposure. The Schumann rays were produced by a discharge tube which was so made that it could be placed under the stage of a compound microscope in the position usually occupied by the condenser and other sub-stage attachments. When the discharge tube was in place, its fluorite window, through which the Schumann rays were emitted, was flush with the microscope stage. The Schumann light shone upward toward the microscope objective. The organisms were exposed above the discharge tube on a special microscope slide which contained a window of fluorite (glass is opaque to these rays). The regular sub-stage attachments could then be swung into place and the organisms observed under high magnifications.

The effects produced by the light were immediate. There was a marked stimulation, followed by cytolysis, which, with a sufficient exposure, terminated in death. All of these changes were usually produced by an exposure of less than one minute's duration.

It was found that a given amount of exposure to the light produced the same effect whether the exposure were continuous or interrupted. This made it convenient to interrupt the exposure from time to time and to make a detailed study of the progress of the changes produced by the light.

The temperature of the drop of water containing the organisms was measured by means of a thermal junction, the variable junction of which was placed beside the organisms under the coverglass. As the temperature did not rise 1° C. during the experiment, the changes produced could not have been due to heat.

The length of time required for killing varied both with the species and with the individual organisms. In general, a small

organism was killed more quickly than a large one. With a given intensity of light, an exposure of several minutes was not sufficient to kill such organisms as rotifers and minute worms, while *Sphaerella*-like swarmspores, which contain both chlorophyll and an "eye-spot," were killed almost instantly. The swarmspores were killed so quickly that there was not sufficient change in temperature to be indicated by the thermal junction. The protoplasm of the swarmspores which had been killed by the light had a granular appearance. Often some of the protoplasm was extruded from the cell and was rounded up into drops.

The cells of a large *Spirogyra* of the *crassa* type were killed by an exposure of 45 seconds when the discharge tube was carrying 18 milliamperes. The various cell organs were affected quite differently by the exposure to the light; for instance, the nucleus became enlarged, while the chlorophyll bands contracted about it and became disorganized.

Active amoebae often showed very marked negative phototropism. The tips of their extended pseudopodia usually turned upward away from the light. Often a pseudopodium was pushed up from the upper surface of the body. The nucleus, together with a large portion of the granular endoplasm, flowed into this pseudopodium, leaving a clear ectoplasm below. With an amoeba in this condition, a properly timed exposure killed all of the lower part without killing the upper part; so that after the exposure, the protoplasm contained in the vertical pseudopodium moved away, leaving the coagulated lower part behind. In some cases, so much of the protoplasm flowed up into the pseudopodium that the amoeba became too heavy and toppled over. One amoeba was seen to send up a pseudopodium, to fall over, and then to repeat the process three times before it was killed.

Under the influence of the Schumann rays the endoplasm contracted, so that there appeared to be an increase in the amount of ectoplasm. The line separating the endoplasm from the ectoplasm was sharply defined. After a prolonged exposure, there was often a peculiar flowing of the granular endoplasm out into the ectoplasm. It did not appear to be the same kind of motion which one observes in the regular streaming of the protoplasm, but it was not easy to

say wherein the difference lay. After this all motion ceased and the protoplasm appeared coagulated. Under a high magnification (2200 diameters) the protoplasm was seen to be filled with small vacuoles which were so numerous that it had the appearance of a fine froth. These vacuoles were not visible before the organism was exposed to the light.

The length of exposure necessary to bring about these changes varied from 30 to 100 seconds when the hydrogen discharge tube was carrying 29 milliamperes. Since the effect on the organisms is additive, the entire exposure was not made at one time, but at intervals, so that the experiment often extended over an hour. Thus the changes produced by the light could be more carefully observed.

Infusoria are very quickly cytolyzed by the rapid vibrations of these ultra-violet rays. The nature of the cytolysis varies greatly with the species, and, in some of the minor details, it varies with different individuals.

The writer has observed three kinds of photo-cytolysis in ciliated infusoria: first, a cytolysis which is accompanied by the formation of vesicles on the surface; second, a cytolysis in which some of the internal portions of the protoplasm coagulate; and, third, a cytolysis in which some of the protoplasm disintegrates directly. The first two types of cytolysis were observed in *Colpoda*-like forms, and the third type was observed in *Stylonychia*.

The cytolysis by vesicle formation requires an exposure of about 30 seconds when the discharge tube is carrying 18 milliamperes. The vesicles are filled with a clear liquid and are often as large as the organism itself. Several vesicles may form and again disappear during the exposure. With sufficient exposure, the surface which separates the protoplasm from the fluid contained within the vesicle breaks, and the protoplasm flows out into the vesicle. A still longer exposure may cause the outer wall of the vesicle to rupture. The protoplasm then flows out into the surrounding water, with which it is miscible.

In the cytolysis in which parts of the protoplasm coagulate, an exposure of a few seconds results in the formation of small masses of coagulum, which are at once extruded by the organism. Con-

tinued exposure causes these coagulated masses to form faster than they are extruded. Soon a swelling appears, which bursts, and the protoplasm flows out.

In *Stylonychia*, the protoplasm disintegrates directly, and becomes miscible with the surrounding water. If the current of the discharge tube is increased to 60 or 70 milliamperes the disintegration begins at once. The infusorian darts across the field, leaving a trail of its cytolyzed protoplasm behind. The organism continues to move until only a few cilia with an attached mass of protoplasm is left intact, and this cytolyzes the instant motion ceases.

When thin-walled fungous spores are exposed to the light, the protoplasm either takes on a coagulated appearance, or the spore bursts. The spores which burst explode with such force that they are shot backward by the reaction. After the explosion a small mass of coagulated protoplasm is seen lying near the exploded spore.

This brief description of the visible effects of the Schumann rays is sufficient to indicate that these ultra-violet light rays have a most violent effect upon protoplasm. The writer has demonstrated by methods not described in this paper that the effect of the light is upon the organism itself and not upon the surrounding medium.

Vesicle formation and the bursting of spores point strongly to changes in osmotic relations or in imbition, which may be connected with the fact, as shown in a previous paper,[4] that the longer ultra-violet light waves have the power to break down proteins. It will undoubtedly be found that these rays have a similar, and, judging from the violence of their action, a much greater power.

Laboratory of Plant Physiology
Harvard University

[4] Science N.S. 37:24. 1913.

FLOWER OF ADENOCAULON BICOLOR

Jessie A. Ayres

(WITH PLATES XI AND XII)

Adenocaulon bicolor Hook. is distributed from the Himalaya Mountains to Japan, and from the northwestern part of the United States to Lake Superior. The only other species is *A. chilenae* Less., from Chile to the Straits of Magellan. The plant is a peculiar one, having no pappus, but an abundance of glandular hairs on the seed. This work was undertaken under the direction of Dr. T. C. Frye of the University of Washington, with the object of comparing the development of the staminate and pistillate flowers.

The heads are arranged in a raceme (fig. 1), which appears as a swelling between the upper leaves (fig. 2, *s*). On this swelling cycles of protuberances appear (fig. 3, *a*, *c*), which become the bracts of the inflorescence subtending the peduncles; but the uppermost whorl becomes the involucre of the terminal head (*c* in figs. 4–9). When the involucre arches over the head, the primordia of the individual flowers appear as bulges on the receptacle (figs. 6 and 7, r^1, r^2). Some of the primordia divide, thus increasing the number of flowers (figs. 8–10, *x*). This mode of increasing the number of flowers suggests a tendency in the heads to branch, and points toward a probable ancestral form with scattered flowers. The axillary heads appear shortly after their bracts arise (fig. 9, *b*). The flowers develop in acropetal succession, as do the parts of the individual flowers (fig. 10).

In the staminate flowers, when the floral parts begin to develop, the flower primordia first broaden at the top (fig. 9, *p*). The corolla then appears as a marginal ring (fig. 10, *mr*) on the top. After the corolla tube lengthens and begins to curve inward, a ring of small bulges appears in the throat of the tube; these are the beginnings of the stamens (fig. 10, *st*). When the stamens are well started, the carpels appear beneath them (fig. 11, r^2, *ca*). Thus all the normal parts except the calyx are present (fig. 12, r^2). When the bundle enters the staminate flower, it separates into a

whorl of five strands about a central strand. The latter terminates at the base of the sterile ovarian cavity; while the five strands about it pass up about the ovarian cavity until they reach half of its length (fig. 13, b^1). Then the two of these strands which are opposite to the two lobes of the stigma branch (fig. 13, b^2), each branch passing up into a lobe of the stigma. The main branch of each of these two strands and also the three other main strands pass up into the corolla, where each branches just below the base of its opposite stamen (fig. 13, b^3); one branch passes up into the stamen, and the main one continues up into the apex of the corolla. The fact that the stigma is cleft does not agree with descriptions in ENGLER and PRANTL'S *Die natürlichen Pflanzenfamilien*, BRITTON and BROWN'S *Illustrated flora*, and HOWELL'S *Flora of Northwest America.*

In the pistillate flower the history is the same up to the development of the ovarian cavity. When the base of this cavity broadens (fig. 12, r^1, *oc*), a bulge appears, which is the beginning of the ovule.

The megaspore mother cell is formed in the usual way, and occupies all or nearly all of the outer end of the nucellus. Behind the megaspore there is crowded a row of two or three other rather large cells, which gives the appearance of a row of three or four megaspores. Sometimes other cells remain at the side of the mother cell, and the outermost ones are often elongated and resemble the mother cell in size but not in content (fig. 14, *nu*). The megaspore mother cell passes through the usual two successive divisions, and the inner megaspore becomes the embryo sac (fig. 21). The embryo goes through the same phases of cleavage (fig. 22) as those reported by MERRELL[1] in *Silphium*.

When the megaspore mother cell has enlarged (fig. 14), the first gland-hairs appear on the akene. Usually the first glands appear just where one would expect a calyx. This leads one to suspect that the tendency of the cells at that point to form projections is still slightly more marked than at other points. These glands develop from a protrusion of four epidermal cells of the akene. These cells are large and have large nuclei (fig. 15). They elongate and form a knob on the end (figs. 15–19). Many more glands

[1] BOT. GAZ. 29:115–124. 1900.

appear shortly after the first ones. In the mature plant the whole inflorescence and the upper part of the stem is glandular, but no glands appear at all on the staminate flowers.

The paths of the axial bundles are the same as those in the staminate flowers, with two exceptions: first, the strand in the center of the whorl of five strands at the base of the ovarian cavity is not abortive, but passes into the ovule and curves with the integument to a point level with the antipodal cells (fig. 20, *a*); second, even when there are stamens no branch of the upper corolla strand branches into them. After fertilization, the style and corolla wither and drop off. When this happens, the whorl of five strands which lies adjacent to the integument (fig. 20, *b*) disorganizes and disappears, along with tissues which are also adjacent to the integument, thus freeing the ovary from the integument.

The style is cleft (fig. 20, *sti*) and the stigma is covered with papillae like those on the staminate flowers.

When the ovarian cavity begins to develop, the growth of the stamens is retarded. Usually they disappear, but sometimes remain as seen in fig. 20, *st*, but none bearing pollen sacs were seen.

There was nothing unusual found in the formation of pollen grains. The outer wall layer becomes thick and spiny, while the spore becomes winged. In grooves between the wings, the extine is merely in contact. At the three germinative spots the walls are pushed outward (fig. 23). This occurs before the pollen grains leave the anther.

Summary

The development of the staminate and pistillate flowers is the same up to the development of the ovarian cavity.

Both staminate and pistillate flowers have ovarian cavities, but ovules develop only in the pistillate flowers.

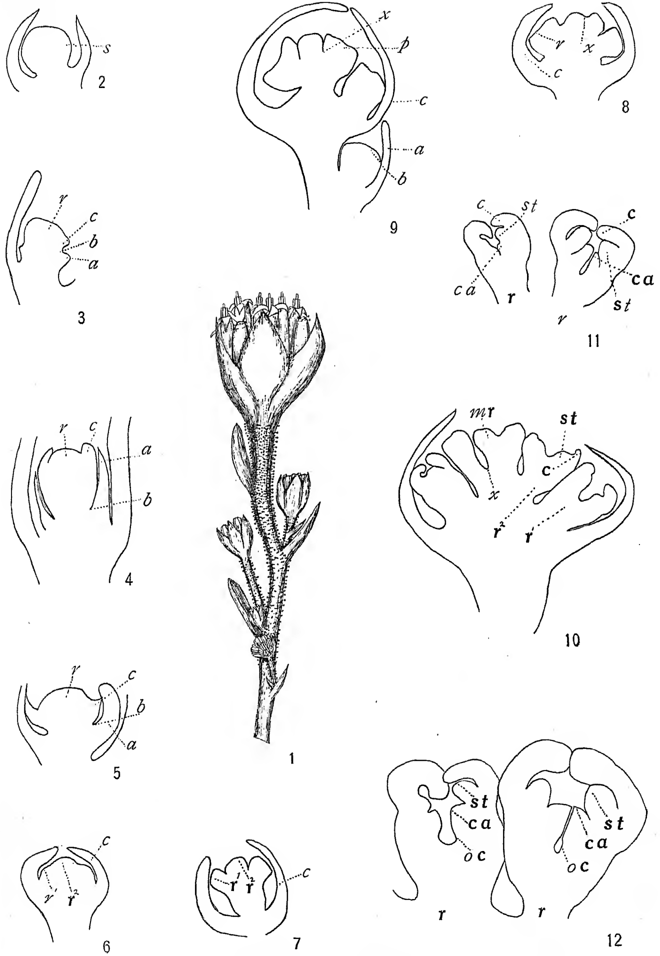
Stamens are sometimes found in pistillate flowers, but they are always sterile.

No gland-hairs are found on staminate flowers.

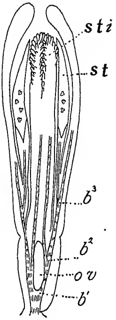
Styles of staminate flowers are cleft.

Nothing unusual occurs in the development of the egg, embryo, or pollen grains.

UNIVERSITY OF WASHINGTON
SEATTLE, WASH.



AYRES on ADENOCAULON



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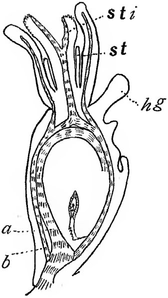

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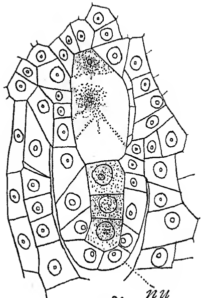

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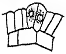

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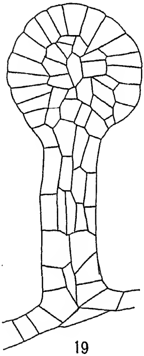

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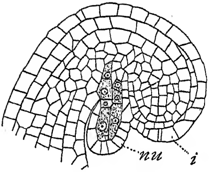

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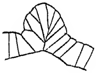

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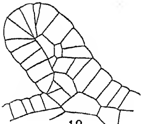

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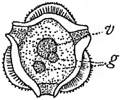

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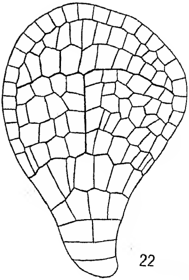

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AYERS on ADENOCAULON

EXPLANATION OF PLATES XI AND XII

All figures except fig. 1 were made with a Bausch and Lomb camera lucida. The plates are reduced to one-half of the original size. The magnifications are for the drawings as reduced.

PLATE XI

All figures (except fig. 1) ×77.5

FIG. 1.—A mature inflorescence; ×8.

FIG. 2.—The first appearance of raceme between upper leaves (*s*).

FIG. 3.—First appearance of bracts of raceme (*a* and *c*); *b*, axis of bract; *r*, receptacle of terminal head.

FIGS. 4 and 5.—Further development of raceme.

FIG. 6.—First appearance of flower primordia (r^1 and r^2).

FIG. 7.—Further development of flower primordia.

FIG. 8.—A primordium dividing (*x*).

FIG. 9.—Further division of primordium (*x*); *p*, widening of top of staminate flower; *c*, involucral bract; *b*, axillary head; *a*, bract of the inflorescence.

FIG. 10.—Inflorescence completed: *x*, division of a primordium almost completed; r^2, staminate flower; r^1, pistillate flower; *mr*, first appearance of corolla by a marginal ring; *c*, corolla in more advanced stage; *st*, stamen.

FIG. 11.—Further development of staminate (r^2) and pistillate (r^1) flowers: *c*, corolla; *st*, stamens; *ca*, carpels.

FIG. 12.—Further development of fig. 11: *oc*, ovarian cavity; other parts labeled as in fig. 11.

PLATE XII

FIG. 13.—A developed staminate flower: *sti*, stigma; *st*, stamen; *oc*, ovarian cavity; b^1, b^2, and b^3, branching points of the bundles; ×30.

FIG. 14.—Ovule: *i*, integument enveloping megaspore mother cell; a cell of the nucellus still remaining parallel to the megaspore mother cell; *nu*, nucellus; ×335.

FIG. 15.—First appearance of gland-hairs by the elongation of epidermal cells; ×335.

FIGS. 16–18.—Development of gland-hairs; ×335.

FIG. 19.—Developed gland; ×250.

FIG. 20.—A mature pistillate flower showing path of the bundles: *sti*, cleft stigma; *st*, sterile stamen; ×30.

FIG. 21.—Inner megaspore has become the embryo sac, and its nucleus has passed through the first stage of division; *nu*, nucellus; ×766.5.

FIG. 22.—Older embryo; ×550.

FIG. 23.—Details of the extine and intine: three germination spots; *v*, vegetative nucleus; *g*, two male cells; ×766.5.

CURRENT LITERATURE

MINOR NOTICES

Submerged forests.—People invariably are fascinated by evidences of former forests below present sea-levels, as indicated by the appearance at low tide of stumps *in situ*. That these "Noah's woods" are of large scientific interest is well brought out in an interesting little booklet by CLEMENT REID, forming one of the useful series of "Cambridge Manuals of Science and Literature."[1] REID speaks in detail of the various submerged forests of the English coast and of the Dogger Banks in the North Sea. The lowest of these forests are oak forests much like those of the present day, though apparently with fewer species. Among the species absent are the more southern types that have come into England since the ice age; at the same time, the glacial relicts have disappeared. It is concluded that the lowest of these forests date back about 5000 years, and that there has been no essential change of sea-level in the last 3500 years. During the period of the life of these forests the climate was much like that of the present, and the land was 60–90 feet higher than it is today.

In connection with REID'S book on submerged forests, attention may be called to a short paper by OSBORN, dealing with a submerged forest on the coast of Wales.[2]—H. C. COWLES.

Plant evolution.—CAMPBELL[3] has presented an interesting popular account of plant evolution for the "American Nature Series." Following a brief consideration of the factors in evolution, the different plant groups are considered, from the bacteria and blue-green algae through the angiosperms, the object here being to set forth the chief characters of each group in the scale of ascent. The book closes with four chapters having the following titles: environment and adaptation, the problems of plant distribution, the human factor in evolution, and the origin of species. Since most books of this character deal with animals rather than with plants, this volume of CAMPBELL'S is especially welcome.—H. C. COWLES.

[1] REID, CLEMENT, Submerged forests. pp. 129. *figs. 4* and *frontispiece*. Cambridge University Press. 1913.

[2] OSBORN, T. G. B., A note on the submerged forest at Llanaber, Barmouth. pp. 10. *figs. 2. pls. 2.* Reprint from Mem. Proc. Manchester Lit. Phil. Soc. 56: 1912.

[3] CAMPBELL, D. H., Plant life and evolution. 8vo. pp. iv+360. *figs. 22*. New York: Henry Holt & Co. 1911. $1.60.

NOTES FOR STUDENTS

The vegetation of Connecticut.—NICHOLS[4] has published the first three papers of a contemplated series detailing the various ecological features of the vegetation of Connecticut. The first of these papers deals with general phytogeographic features, both floristic and ecological. The most interesting floristic problem considered is the segregation in the southeastern part of the state of many characteristic coastal plain plants. Some of these plants are confined to the southeastern part of the state, and the rest are decidedly more frequent there. One of the most important of the latter class is *Chamaecyparis thyoides*, the chief character plant of the "cedar swamps"; associated with this is *Rhododendron maximum*. Since these coastal plain plants are found more or less continuously along Long Island, NICHOLS favors the theory of a postglacial land bridge connecting eastern Long Island with the mainland, as suggested by HOLLICK. The climax forest of the state is very mesophytic and is composed of several deciduous trees (chestnut, white oak, red oak, sugar maple, red maple, beech, tulip, linden, etc.) with the hemlock. The forests in the hilly regions of northwestern Connecticut contain the spruce, fir, and other forms that are strikingly more boreal than are the coastal plain plants of southeastern Connecticut. After giving brief consideration to maritime and some other associations, NICHOLS notes a few of the cases of eccentric distribution in the state; a notable instance is that of a moss, *Claopodium pellucinerve*, which is known elsewhere only from the Yukon Territory and India. The first paper closes with an account of the climate and physiography of the state.

The second paper is devoted to a consideration of the virgin forests of Connecticut. Much the finest of these was the recently destroyed Phelps forest in Colebrook, in the northwestern part of the state. So far as known, this forest of 300 acres dates back to prehistoric days, almost unmodified by the ax or by fire. It is taken to be the dominating type of forest in the state, up to the time of settlement by the white man, and may be regarded as the best example of a climax forest in Connecticut. *Fagus grandifolia* and *Tsuga canadensis* make up 55 per cent of the stand. *Acer saccharum* and *Betula lutea* comprise 22 per cent. The remaining 23 per cent is made up of *Quercus rubra*, *Castanea dentata*, *Fraxinus americana*, *Tilia americana*, *Prunus serotina*, *Betula lenta*, *Acer rubrum*, and *Pinus Strobus*. Some of the trees are of immense size, and it is noted that the same species form the undergrowth. There is a rich undergrowth of shrubs, in which a large part is played by *Taxus canadensis*, *Viburnum alnifolium*, and *Kalmia latifolia*. The liverwort and moss flora are astonishingly rich. It is a matter of profound regret that this beautiful and unique forest was destroyed in 1912, so that the last extensive primeval woodland of the state has gone. NICHOLS is to be congratulated upon having made a record

[4] NICHOLS, G. E., The vegetation of Connecticut. I. Phytogeographical aspects. II. Virgin forests. III. Plant societies on uplands. Torreya **13**:89–112. *figs. 6;* 199–215. *figs. 5.* 1913; **14**:167–194. *figs. 9.* 1914.

of its plant population before it was too late. Three fragments of primeval forest are noted in the same part of the state. The population is much the same as that of the Colebrook forest, except that the one in Cornwall is dominated by *Pinus Strobus;* these pines are said to be the most magnificent still existing in the East. The pine is not the climax type, however, since the undergrowth is dominated by hemlock and several hardwoods.

In a third paper, NICHOLS details the successional relations of the upland vegetation of the state. One of the most characteristic rock types of Connecticut is the trap ridge, whose vegetational history is depicted from the pioneer crustose lichens through the herb, shrub, and tree stages. Early trees are *Juniperus virginiana* and *Quercus stellata*, following which *Carya glabra* and *Quercus Prinus* are likely to prevail. These form a somewhat open forest, which later develops into a closed forest, dominated by several species of oak and hickory. This or a more mesophytic forest represents the climax type. The trap ridges have very characteristic talus slopes, on which the vegetation passes rather rapidly to the mesophytic climax forest. Another interesting upland succession is that of the sand plains, on which one of the prominent early tree stages is dominated by *Pinus rigida*, which later is succeeded by oaks. In the state are many abandoned fields which are reverting to forest, an early stage being that dominated usually by *Betula populifolia*, *Juniperus virginiana*, and *J. communis depressa*. Note is made of the varying importance of certain trees in different portions of the state; *Pinus Strobus* is of special interest in this respect, being a common pioneer in some parts, almost a climax tree in other parts, and negligible in still other parts. The importance of the chestnut in many Connecticut woodlands is being greatly lessened, owing to the ravages of the chestnut bark fungus, *Endothia gyrosa parasitica*.—H. C. COWLES.

Inheritance of semi-sterility.—BELLING[5] has made a careful analysis of the inheritance of partial sterility in crosses of *Stizolobium deeringianum* Bort. (Florida velvet bean) with other species of the same genus, namely *S. niveum* (Roxburgh) Kuntze (Lyon bean), *S. hassjoo* Piper and Tracy (Yokohama bean), and *S. niveum* var. (China bean). The pollen from healthy flowers of all these forms was found to be nearly 100 per cent good. A few ovules of some pods only were found to have aborted, due, the author believes, to circumstances unfavorable for those particular pods. From a third to a half of the pods had no aborted ovules. The ovules in general had completely formed embryo sacs. Of the F_1 generation of the three crosses, approximately 50 per cent of the pollen grains were found to be shrunken and non-viable, the other 50 per cent being perfectly developed and viable. Similarly, approximately 50 per cent of the ovules of F_1 plants abort. Sections of young ovaries

[5] BELLING, JOHN, The mode of inheritance of semi-sterility in the offspring of certain hybrid plants. Zeitschr. Ind. Abs.- u. Vererbungs. 12:303–342. 1914.

showed almost exactly one-half of the ovules with complete embryo sacs "nearly filling the nucellus," while the other ovules, "though full sized, had either quite aborted embryo sacs, the nucellus being a uniformly cellular mass, in which, however, the remains of the aborted megaspore could usually be distinguished, or had only a minute cavity to represent the embryo sac." The fact that somewhat less than half the seeds of ripe pods were found to have aborted is believed to be due to a selective elimination through dropping of young pods that chanced to have all or nearly all aborted ovules. In the F_2 generation about one-half of the plants had almost wholly normal pollen grains and in the other half approximately 50 per cent of their pollen grains aborted as in F_1. The F_2 plants with normal pollen also had few aborted ovules just as the parent species had, while those with semi-sterile pollen had about 50 per cent aborted ovules. The F_3 progenies of fertile F_2 plants were wholly fertile. The semi-sterile F_2 plants, like all F_1 plants, produced F_3 offspring a half of whose members were fertile and a half semi-sterile with regard to both pollen and ovules. Some of the fertile F_3 stocks were grown on a large scale and found to breed true to that characteristic in F_4 and F_5.

The author points out clearly that the form of sterility observed in *Stizolobium* hybrids manifests itself only in the haploid, never in the diploid generation. The F_2 ratio of 1:1 from selfed F_1 plants is not, therefore, a zygotic but rather a gametic ratio. It is interpreted accordingly as simple Mendelian segregation, exceptional only in that the segregation is here visible in the gametes themselves and not merely inferred from the appearance and behavior of the zygotes as in ordinary Mendelian cases. Since gametes are haploid (simplex for genetic factors), it is obvious that dominance and recessiveness, either complete or partial, cannot in any way be concerned in this problem. As a working hypothesis, the author assumes two genetic factors, *K* and *L*, one present in *S. deeringianum* and the other in each of the other species. It is assumed that the presence of one or the other of these factors is essential for the development of normal pollen grains and embryo sacs, but that in the presence of both factors, just as in the absence of both, no development results. The hypothesis accounts for all the facts observed. The author promises the further crucial test of crossing together different fertile lines from the progeny of semi-sterile plants, in which, if the hypothesis holds, half of the crosses should yield only fertile and half only semi-sterile offspring. It would be instructive also to cross together the three forms other than *S. deeringianum*, all of which should produce wholly fertile hybrids.

It remains only to be said that, as an alternative hypothesis, *K* and *L* might be regarded as inhibitors of complete gametic development when present singly, the one neutralizing the effect of the other when both are together. *KL* and *kl* would then result in normal and *Kl* and *kL* in aborted gametes, just the reverse of the author's assumption. Both assumptions are equally in accord with the observed facts and the same further results are to be predicted from both. The author is to be congratulated upon the thoroughness of his

analysis and the clearness of its presentation. The excellent illustrations accompanying the paper add not a little to its effectiveness.—R. A. EMERSON.

Calcicoles.—MALCOLM WILSON[6] has made a study of the varying composition of the woodlands of southeastern England, in connection with variations in the substratum. His conclusions are in harmony with those of most English ecologists, namely that the flora of the chalk and of other calcareous strata differs considerably from that overlying non-calcareous strata. However, the vegetation on the siliceous London clay differs considerably from that on the very similar "clay with flints," whereas the latter has a vegetation much like that of the chalk. Parallel species are found on the chalk and the clay with flints, the former being more xerophytic in structure; these results agree with those found long ago by KERNER. WILSON shows how other factors, such as depth of soil and amount of shade, are as likely to be limiting factors as is soil composition. These woodlands are largely coppiced every fourteen years or thereabouts, and WILSON pays large attention to the changes brought about at coppicing, through the admission of light, and to the gradual changes later on, as shade increasingly returns. While shade-tolerant species gradually get the upper hand in the years following coppicing, it is interesting to note that certain perennial species, usually regarded as light-requiring, may remain through the shade period; these plants are dwarf in habit and reproduce only vegetatively.

As is well known, most American ecologists place little emphasis on the division of plants into calcicoles, silicicoles, etc. BUTTERS,[7] however, records from the Selkirk Mountains of British Columbia some observations that harmonize well with the calcicole theory proposed by UNGER in 1836. One of the lateral moraines of the Sir Sandford glacier is composed chiefly of fragments of limestone and dolomite, whereas the other lateral moraine is composed chiefly of fragments of granite and mica schist. The flora of these two moraines is strikingly different, only 34 of the entire 110 species occurring on both moraines; only 21 species occur somewhat equally in the two habitats. The flora of the limestone moraine is composed of species that are largely rare or local in the Selkirks, and it is to be noted also that limestones are similarly infrequent in these mountains. All other limiting factors seem excluded except that of difference in chemical composition of the substratum. There are 34 species on these moraines, which were found in eastern North America by FERNALD;[8] 20 of these have exactly the same type of soil distribution in these widely separated regions, and in no case is there a reversal of soil preference.

[6] WILSON, MALCOLM, Plant distribution in the woods of northeast Kent. I. Ann. Botany 25:857–902. *figs. 4. pls. 3.* 1911.

[7] BUTTERS, F. K., Some peculiar cases of plant distribution in the Selkirk Mountains, British Columbia. Minn. Bot. Studies 3 and 4:313–331. *fig. 1.* 1914.

[8] See BOT. GAZ. 45:138–139. 1908.

For fifty years, commencing with KERNER, many European botanists have called attention to the relatively xerophytic features of limestone plants. HOSSEUS[9] shows that these same features hold for the tropics. His studies were made on a mountain in northern Siam, and he records the following xerophytic habits as characteristic: shortened, lignified, much-branched stems; reduced leaf surfaces; involute leaves; succulence, etc.—H. C. COWLES.

The chestnut disease.—ANDERSON and RANKIN[10] have published a bulletin upon the chestnut disease which has attracted so much attention. The bulletin brings together the scattered data in reference to the disease and presents the known facts in a very convenient form. It seems that this "canker" was first discovered by MERKEL in 1904 on the American chestnut in the New York Zoological Park. The rapidity of spread has been phenomenal, and the authors state that "the completeness of destruction is without parallel in the annals of plant pathology." The latest published information states that the disease is now generally distributed among native chestnuts from New Hampshire and the Hudson region of northern New York to Virginia; and has spread westward into New York and Pennsylvania, but has not yet been found in Ohio or Indiana.

The name of the causal organism has been under considerable discussion, and the various views are presented. The authors adopt *Endothia parasitica* (Murr.) Anders. The morphology is discussed in detail, treating of stromata, pycnidia, pycnospores, perithecia, asci, ascospores, and mycelium. It is obvious that the American chestnut (*Castanea dentata*) is by far the most susceptible host, but no species of *Castanea* has been proved to be immune, although some of the oriental varieties show a certain amount of resistance. The conclusion at present is that this disease is not a serious menace to any forest tree except the chestnut. The problem of dissemination is discussed in detail, including such factors as man, insects, rain, birds, wind, and other minor agencies.

Naturally the subject of control is discussed with all available data, and the general conclusion is reached that "at present we know of nothing that will prevent the extermination of the American chestnut tree." The authors, however, "do not believe that the ingenuity of our scientists has been exhausted," a hopeful belief which we trust will be justified.—J. M. C.

Morphology of Peperomia hispidula.—JOHNSON[11] has made a detailed study of this species, having a very simple vegetative structure and a peculiar

[9] HOSSEUS, C. C., Edaphische Wirkungen des Kalkes auf die Vegetation tropischer Karren und Karrenfelder. Bot. Jahrb. 45:661–669. 1911.

[10] ANDERSON, P. J., and RANKIN, W. H., *Endothia* canker of chestnut. Cornell Univ. Agric. Exp. Station Bull. 347:533–618. *pl. 37. figs. 101.* 1914.

[11] JOHNSON, DUNCAN S., Studies of the development of the Piperaceae. II. The structure and seed-development of *Peperomia hispidula.* Amer. Jour. Bot. 1: 323–339, 357–397. *pls. 36–38, 41–43.* 1914.

type of embryo sac, as a basis of comparison with the other species to be described later. His topics are as follows: habit and vegetative structure, development of the spike and flower; the stamen, microspore, and pollen tube; the carpel and fruit, ovule and seed; the embryo sac, embryo, and endosperm; germination; and abnormal embryo sacs.

It is a very full and satisfactory presentation of the facts, on the basis of which the following conclusions are reached. In vegetative structure, *P. hispidula* is simpler than any of its relatives, and its delicate herbaceous stem, as well as the accompanying structures, are probably due to recent modification of the more complex type of structure. The flowers are naked, and there is no evidence that they ever possessed a perianth. The megaspore mother cell develops a tetrahedral tetrad of megaspores, whose delicate walls soon disappear, leaving the 4 nuclei in a continuous protoplast. The 4 megaspore nuclei divide to form the characteristic 16-nucleate embryo sac, with its egg and solitary synergid, and its huge endosperm nucleus, formed by the fusion of 14 nuclei. This embryo sac cannot be regarded as primitive, but rather as a compound sac, a structure unknown among the simpler forms. The restriction of the function of the endosperm to that of a "nurse" for the embryo, the food supply being stored in the perisperm, is regarded as "the next to the last step in the disappearance of the endosperm," which becomes practically complete in the Helobiales, Orchidaceae, etc.

To the reviewer, this study is a most satisfactory illustration of the fact that many conditions which appear primitive upon superficial examination may prove upon real examination to be derived and specialized conditions. —J. M. C.

Peridium formation in the aecium.—KURSSANOW[12] adds several interesting details to former accounts of the process by which the layer of peridial cells is formed over the outer surface of the developing mass of aeciospores. It had formerly been held that no intercalary cells were formed in the peripheral chains that constitute the lateral walls of the peridium, and that the peridial cells that make up these chains were metamorphosed aeciospore initial cells that had failed to divide. KURSSANOW finds, however, that intercalary cells are normally produced in these peripheral chains as well as in the interior aeciospore chains. They are not intercalary in position, but are cut off at the lower outer corner of the initial cell when this cell is about the third from the base of the chain. They enlarge somewhat after their abstriction, but soon become disorganized and form a structureless, gelatinous layer between the outer wall of the peridium and the sterile tissue that surrounds it. The production of intercalary cells was more readily followed and the cells were more persistent in the deep-seated, cylindrical aecia of *Puccinia graminis* and *Gymnosporangium tremelloides* than in any of the other 8 species with cupulate

[12] KURSSANOW, L., Über die Peridienentwicklung im Aecidium. Ber. Deutsch. Bot. Gesells. 32:317–327. *pl. 6. figs. 2.* 1914.

aecia that were studied. In agreement with others he finds that the cells of the central arch of the peridium are the apical cells of the central spore chains that have, before their metamorphosis into peridial cells, cut off intercalary cells below. All of the cells of the peridium are therefore morphologically aeciospores. An apparent exception to this was found in *Peridermium Pini*. In the division of the peridium initial cells of the central arch the usual process is reversed and the small intercalary cell is cut off above and the peridial cell below. A brief description of the fertilization processes in this species is given. Equal cell fusions similar to those first described by CHRISTMAN were found.—F. D. FROMME.

Reciprocal crosses of Oenothera.—DAVIS[13] has reported a partial confirmation of the results obtained by DE VRIES from reciprocal crosses between *Oenothera biennis* L. and *O. muricata* L. The observations of DAVIS also include reciprocal crosses between *O. biennis* L. and *O. franciscana* Bartlett, between *O. biennis* and *O. grandiflora* Solander, and between *O. muricata* L. and *O. gigas* De Vries. Detailed, parallel descriptions are given of the parents and of the pairs of reciprocals, together with numerous photographs of the plants in various stages of their growth. Except in the case of the *gigas-muricata* crosses, the reciprocals of which were in general without important distinguishing characters, the reciprocal crosses exhibited striking contrasting differences. In most respects the crosses closely resembled the pollen parent (patroclinous), as had been noted earlier by DE VRIES for one of these crosses, but strong matroclinous tendencies were also observed, particularly in certain features of the inflorescence of the *biennis-muricata* crosses. Red coloration was found to be wholly or partially dominant without respect to whether it was contributed by the paternal or maternal parent. Moreover, in all the crosses observed by DAVIS, even where patroclinous and matroclinous tendencies were most conspicuous, the influence of both parents was plainly recognizable. He has "observed no certain evidence that a morphological character of either species in a cross is passed on to the F_1 hybrids exactly as it is represented in one or the other of the parents." This fact, DAVIS notes, would render untenable GOLDSCHMIDT'S assumption of merogony, even though that explanation had not been made doubtful by the cytological data of RENNER. No satisfactory explanation of these results has been suggested.—R. A. EMERSON.

Transpiration in succulent plants.—DELF[14] has made an interesting study of the transpiration peculiarities of the different classes of succulent plants, having carried on a number of experiments and having endeavored to organize

[13] DAVIS, BRADLEY MOORE, Genetical studies on *Oenothera*, V. Zeitsch. Ind. Abst.- u. Vererbungslehre 12:169–205. 1914.

[14] DELF, E. MARION, Transpiration in succulent plants. Ann. Botany 26:409–442. 1912.

in a systematic way the very chaotic literature of the subject. It is concluded that the chief structural features of these plants are connected with the transpiring surface and the accumulation of water. As to the transpiring surface, there is a greater or less amount of reduction, supplemented in many cases by features that tend to diminish transpiration, such as protected stomata, aerial water absorption, wax coats, etc. The formation of the water tissue that is so characteristic of succulents seems to be "related to the production of organic acids, owing to the influence of limited gaseous exchange on metabolism, and to the presence of chlorides or sulphates in excess in the soil water." DELF agrees with HOLTERMANN that these considerations do not fully explain succulence, since some plants (as *Salicornia*) are so far modified as to be obligate halophytes, whereas other plants (as *Aster Tripolium*) are facultative halophytes, and still others (as *Suaeda fruticosa*) can endure either saline or non-saline habitats without appreciable structural change. In some cases succulence is a hereditary feature, whereas in others it is related to the conditions experienced by the individual showing it. The author believes that water tissue in all cases is of advantage in allowing a plant to "support a rate of water loss which is very considerable, relative to the transpiring surface."—H. C. COWLES.

The vegetation of Clare Island, Ireland.—A paper by R. L. PRAEGER on the vascular plants of Clare Island is but one of a large series of papers, published as Volume 31 of the Proceedings of the Royal Irish Academy.[15] The total number of papers or parts is 68, thus representing probably the most complete natural history survey ever made of any district in the world. The work has been carried on by more than a hundred specialists. The thoroughness with which the work has been done is well illustrated by the fact that in 18 papers there are recorded nearly 700 species of plants and animals not previously found in Ireland, 60 not previously found in the British Isles, and 17 species that are new to science.

Clare Island is an exposed headland, embracing six square miles, and situated three miles from the mainland. The highest point is 1500 feet above the sea. The number of vascular plants indigenous to the island is under 400. The dominating vegetation type is moorland, which includes practically everything over 200 feet. On the precipitous Croaghmore cliff, 1500 feet high, there is a remarkable alpine colony of 10 species, some of which come down almost to sea-level. There is a detailed and interesting discussion of the origin of the flora. Attention is given to the possibility of a land bridge. Wind and birds are regarded as more important than water as dispersing agents.

[15] PRAEGER, R. L., Phanerogamia and Pteridophyta. Clare Island Survey; a scientific survey of Clare Island, in the county of Mayo, Ireland, and of the adjoining parts of the mainland. Proc. Roy. Irish Acad. 31^{10}: 1–112. *pls. 6.* 1911. The entire series can be secured for 60*s.* from the Secretary, Royal Irish Academy, Dawson St., Dublin.

It is noted that a good deal depends on the efficiency of accidental or occasional dispersal.—H. C. Cowles.

The origin of Monocotyledons by self-adaptation.—A great many years ago Henslow proposed the strange theory that Monocotyledons have arisen from Dicotyledons through self-adaptation to an aquatic habitat. Recently he has published[16] further along similar lines; now, however, he regards the notion as a fact instead of a theory, although his line of reasoning is practically unaccepted and is quite out of harmony with the views of modern morphology and ecology. His argument is based on the unsound premise that such formative reactions as those of amphibious plants to water lie at the root of the evolutionary process. No one knows what lies at the root of the evolutionary process, but it is rather certain that it is not this. Water is regarded as causing degeneracy in form and structure, and aquatic seed plants are regarded as degraded land plants. Monocotyledons are supposed to have arisen from Dicotyledons by such degeneracy; non-aquatic Monocotyledons have merely crawled back again upon the land, though retaining their degenerate features. Other authors have regarded Monocotyledons as degenerate Dicotyledons, but self-adaptation as a cause of degeneracy has rarely been postulated; indeed the two ideas, self-adaptation and degeneracy, to the reviewer seem mutually contradictory. A form that is plastic and becomes suited to its environment should not be called degenerate, even though certain organs are reduced or even lost.—H. C. Cowles.

Anatomy of the node.—Sinnott[17] has concluded that the anatomy of the node may be of great service in indicating the relationships of angiosperms. He considers the "trilacunar" type of node as probably the most ancient available type, meaning that there is a foliar supply of three bundles, each causing a gap of its own in the stem cylinder. This type is characteristic of the Amentiferae, and is present in the majority of Ranales and Rosales. Derived by reduction from this type, as indicated by the study of transitional families, is the "unilacunar" type, characteristic of all the Centrospermae and also of numerous families of the Archichlamydeae and Sympetalae. There is also a "multilacunar" type, derived by the "amplification" of the primitive trilacunar type, which reaches its highest development in Polygonales and Umbellales.

In developing the facts, Sinnott has examined about 400 genera, distributed among 36 orders, and gives a list of families with their number of nodal

[16] Henslow, G., The origin of Monocotyledons from Dicotyledons through self-adaptation to a moist or aquatic habit. Ann. Botany 25:717–744. 1911; see also Jour. Roy. Hort. Soc. 37:88–94, 289–294. 1911.

[17] Sinnott, E. W., Investigations on the phylogeny of angiosperms. I. The anatomy of the node as an aid in the classsification of angiosperms. Amer. Jour. Bot. 1:303–322. *pls. 30–35.* 1914.

gaps. He feels justified in expressing the opinion that nodal anatomy will take an important place in the final construction of the phylogeny of angiosperms.—J. M. C.

Marine algae of Peru.—HOWE[18] has published an account of the marine algae of Peru, based chiefly upon collections made by Dr. ROBERT E. COKER while acting as fisheries expert to the government of Peru during the years 1906–1908. The list includes 96 species, 29 of which are described as new. Among the latter is a new genus of Rhodophyceae (*Lobocalyx*), referred to Nemalionaceae. The distribution among the great groups is as follows: Cyanophyceae 7, Chlorophyceae 20, Phaeophyceae 15, Rhodophyceae 54. The economic importance of the marine algae, recently emphasized by investigations carried on by the United States Department of Agriculture, is referred to in this report. Attention is called to the fact that *Macrocystis* and the other large seaweeds (as *Lessonia* and *Eisenia*) are abundant on certain parts of the coast of Peru, and that they may prove important as a source of "potash."—J. M. C.

Liverworts of Peru.—The Yale Peruvian Expedition of 1911 collected 31 species of Hepaticae in a condition to be identified, 14 genera being represented. Of three thallose species, two belong to Marchantiales. According to EVANS,[19] six species are new: one in *Metzgeria*, four in *Plagiochila*, and one in *Lejeunea* (*Dicranolejeunea*). Apparently all of this material is desiccated and therefore unfit for critical morphological study. It is unfortunate that even at the present day most collectors do not realize the importance of properly preserved material. In the naming of some of these new species "honor" is conferred upon different individuals. It is to be hoped that taxonomists of the future will use descriptive names so far as possible when describing new genera and species.—W. J. G. LAND.

Lepidostrobus.—MRS. ARBER[20] has published an anatomical study of *Lepidostrobus*, which brings together our previous knowledge of the genus and adds some unrecorded features. Perhaps the most noteworthy new feature is the presence of a sterile plate in the sporangia of *L. Oldhamius* and *L. foliaceus*. This delicate radial plate arises from the floor of the sporangium, and dies out toward the distal end. Two new species are described, *L. Binneyanus* and *L. gracilis*, and also two new forms of *L. Oldhamius*.—J. M. C.

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[18] HOWE, MARSHALL AVERY, The marine algae of Peru. Mem. Torr. Bot. Club 15:1–185. *pls. 1–66.* 1914.

[19] EVANS, ALEXANDER W., Hepaticae. Yale Peruvian Expedition of 1911. Trans. Conn. Acad. Sci. 18:291–345. *figs. 11.* 1914.

[20] ARBER, AGNES, An anatomical study of the paleozoic cone genus *Lepidostrobus*. Trans. Linn. Soc. London II. Bot. **8**:205–238. *pls. 21–27.* 1914.

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THE COEFFICIENT OF MUTATION IN OENOTHERA BIENNIS L.

HUGO DEVRIES

The significance of the discovery of the mutability of *Oenothera Lamarckiana*, *O. biennis*, and allied forms is a double one. In the first place, it provides us with material for experimental investigations into the laws which govern the origin of living forms by means of the production of new characters and of the loss of existing ones. The knowledge of such laws must become of the highest practical value as soon as the evidently limited possibilities of producing new forms through the recombination of characters by means of crossing becomes exhausted. This conclusion seems especially well founded, since the old conception of improving agricultural races after the principle of slow and continued selections has now generally been abandoned and replaced by the direct selection of elementary types out of the mixtures which constitute the so-called agricultural races and varieties.

The appearance of really new characters seems to be a very rare phenomenon in nature, and a case in which such changes regularly occur in one or more per cent of all the individuals affords material for experiments, the results of which may be expected to apply to a large series of other species also, including, probably, an important number of agricultural crops.

In the second place, the mutability of the evening primroses has a distinct bearing upon the theory of mutation, or of the origin of all living species from one another by sudden leaps instead of

by slow and almost invisible changes as was assumed by DARWIN. The theory itself does not, of course, depend on this or other single instances; it is founded upon general considerations taken from almost all branches of biological and paleontological research, as I have often pointed out.[1]

One of the main arguments is the statement that adaptations cannot, as a rule, have been produced by slow improvements, and that quite a large number of differentiations in organization, if not almost all the really important ones among them, are not adaptations at all.

Apart from our poetical admiration of nature, we have no other way of judging the reality and efficiency of supposed adaptations than by their effects in the struggle for life. Species which are distributed over large countries and occur in thousands of individuals are evidently well fitted for their life conditions. Newly introduced forms, which are spreading with astonishing rapidity and gaining a large territory often in the lapse of a few years, thereby show the highest degree of adaptation to their new environment. But a showy differentiation may be followed by a wide distribution, as in the case of *Drosera*, or limit the species to a relatively very small area, as in *Dionaea*.

Of late J. C. WILLIS has brought forward the most conclusive evidence against the theory of natural selection and in favor of an origin of species by mutation.[2] He bases some of his arguments upon his observations of the endemic species of Ceylon, such as are found in *Coleus*, *Acrotrema*, and other genera. If these endemics had evolved according to the law of natural selection, in consequence of a gradually increasing adaptation to their local environment, it would follow that they must now be better adapted than their parent types, conquer these in the struggle for life, and become quite common, while the old forms would tend to disappear. As a matter of fact, however, their behavior is quite the contrary.

[1] DEVRIES, HUGO, The mutation theory. 2 vols. 1909–1910; Species and varieties, their origin by mutation, 2d ed., 1906; Die Mutationen in der Erblichkeitslehre. pp. 42. Berlin. 1912; The principles of the theory of mutation. Science 40:77–84. 1914.

[2] WILLIS, J. C., Some evidence against the theory of the origin of species by natural selection of infinitesimal variations, and in favor of origin by mutation. Ann. Roy. Bot. Gard. Peradeniya 4:1–15. 1907.

The endemics are rare, often strictly local, and grow in the midst of a luxuriant vegetation of their widely spread and thriving ancestors. It is hardly necessary to point out that this conclusion holds good not only for Ceylon, but for the origin of endemic and local species in general.

WILLIS has also called attention to the Podostemaceae and the allied group Tristichaceae. They show one of the most interesting illustrations of a very rich differentiation without the least indication of a relation to their environment. A very great uniformity in the conditions of life is combined with a most remarkable variety in their morphological structure. In the Podostemaceae the flowers are anemophilous, terminal, and erect, but combine with these characters of low organization the highest degrees of dorsiventrality and of differentiation, and this without any reference to advantages or disadvantages to be derived from them in their functions. Numerous points of similar significance in the structure of the vegetative and reproductive organs are pointed out by the author. Moreover, the genera *Tristicha* and *Podostemon*, which are widely distributed, are comparatively little modified from the earlier types of the orders, while the highly specialized forms are at the same time the rarest, exactly as in the case of the endemics of Ceylon.[3]

In the group of the evening primroses the same principles prevail. Their struggle for existence is limited by the difficulties which they have in producing roots. Cuttings almost never succeed in rooting, with the exception of the lateral rosettes at the base of the stem. Artificial transplanting becomes difficult as soon as the main root increases in size. In the field only a small percentage of the seeds germinate and thrive, and this only under special conditions. They want a stirred up soil and do not like to grow between other plants. These characters are common to all the forms which I have had an opportunity of studying in their native habitats. On the other hand, the numerous small specific differentiations, such as the form of the leaves, the branching of the stem, or the structure of the flowers and fruits, do not show

[3] WILLIS, J. C., On the lack of adaptation in the Tristichaceae and Podostemaceae. Proc. Roy. Soc. 8:532–550. 1914.

the least relation to their environments. Even the preference for an annual or a biennial behavior, which might seem to be a direct adaptation, does not exhibit any reference to the actual life conditions. The conception of natural selection and of the accumulation of small variations on account of their utility cannot explain the specific and generic differences in this group.

Therefore it seems unavoidable to assume that specific differentiation in the genus *Oenothera* has been produced and is still being produced by small steps, each of which evolved a character at once to its full development, without any reference to the struggle for life. That, besides this process, from time to time new combinations of characters by means of crosses may have given rise to constant hybrid strains, which we have as yet no means of distinguishing from pure species, cannot of course be doubted.

Now, *Oenothera Lamarckiana*, *O. biennis*, and some allied forms are seen to be still in a condition of making, from time to time, such small steps. They are doing this in their natural habitats as well as in experimental cultures, and the variations produced show no relation to the external conditions of their environment or to the method of their culture. On this ground, the claim seems justified that the mutations, directly observed in the primroses, are similar to those which have produced in nature the specific differences and the differentiating characters in this group. If this is conceded, it follows that the analogous processes in other genera, and even in the origin of the larger systematic groups, must be viewed in the same way. This claim, however, has not escaped serious objections.

The main line of these attacks is based upon the vague and double assumption that *O. Lamarckiana* might be a hybrid, and that its hybrid origin might account for its present mutability. These two assumptions are evidently independent of one another and would have to be proven separately. So far as I know, no attempts have been made as yet to prove the second assumption, and no hybrid races have been produced which, from this cause, give rise to phenomena exactly duplicating the mutations of the primroses. And it is evident that so long as such an analogy is only an ardent wish of the critics, the question whether the mutating

primroses are of pure or of hybrid origin is not of paramount importance for the appreciation of the fact of their mutability.

The first attacks on the gametic purity of the mutating forms have been directed only against *O. Lamarckiana*, and at the present time the most prominent adherents of this opinon are DAVIS and RENNER. They try to give proof of a separate hybrid nature for this species on considerations which do not apply to *O. biennis* and the other mutating forms, and concede for these latter a pure origin.[4]

DAVIS based his arguments upon a historical research concerning the origin of *O. Lamarckiana*, and upon his attempts to duplicate this form by crossing others which he assumed to be of pure line.[5] A specimen collected by MICHAUX in the eastern part of the United States, about a century ago, and studied by L. BLARINGHEM, proves our plant to have been a component of the flora of this country, whence LAMARCK obtained the authentic specimen for his original description.[6]

RENNER studied the empty seeds of *O. Lamarckiana*, which constitute over one-half of the whole crop. He brings this phenomenon in connection with the ability of this species to produce twin hybrids, *laeta* and *velutina*, in certain crosses with older types, and assumes that the sexual cells are one-half potential *laeta*, and the other half potential *velutina*. In the normal fertilization of *O. Lamarckiana* this would produce $\frac{1}{4}$ *laeta*, $\frac{1}{4}$ *velutina*, and $\frac{1}{2}$ of the hybrid combination *laeta*×*velutina*. He further assumes that in pure condition both the *laeta* and *velutina* qualities are incompatible with normal development, and that the germs which bear them are doomed to die at an early stage, thereby leaving their seeds empty. Only the combination *laeta*×*velutina* would be fit for further growth, and if we assume that this shows the marks of *O. Lamarckiana*, the constancy of this form would not be in

[4] DAVIS, B. M., Mutations in *Oenothera biennis* L. Amer. Nat. **47**:116. 1913; and Parallel mutations in *Oenothera biennis*. *Ibid*. **48**: 499–501. 1914.

[5] ———, Some hybrids of *Oenothera biennis* and *O. grandiflora* that resemble *O. Lamarckiana*. Amer. Nat. **45**:193–233. 1911.

[6] BLARINGHEM, L., L'*Oenothera Lamarckiana* Ser. et les Oenothères de la forêt de Fontainebleau. Travaux de biologie végétale, dédiés à GASTON BONNIER. Rev. Gén. Bot. **25**²:35–50. 1914; see also my article in BOT. GAZ. **57**:345–360. *pls. 17–19*. 1914.

contradiction with the other hypotheses. If we accept these views, all reasons for supposing a correlation between the splitting phenomenon and the mutability would lose their value, and this latter process would come much nearer to the corresponding changes in *O. biennis* and allied species. The hypothesis, although resting on too large a number of suppositions, would in some sense be a support for the theory of mutation, since it is evidently impossible that these presumed qualities, which are incompatible with life, could have evolved slowly on the ground of their utility in the struggle for existence. Moreover, the hypothesis has no direct bearing on the observed phenomena of mutation, and the fact that in *O. biennis* such empty seeds are wholly or almost wholly absent proves beyond doubt that mutability may be independent of them. Thus the hypothesis of RENNER emphasizes the importance of a study of the mutation phenomena in *O. biennis*, in contradistinction to those in *O. Lamarckiana*, at least for the present, until facts are available to appreciate the correctness of his views.

Obviously the hypothesis that *O. Lamarckiana* might be a hybrid, whilst *O. biennis* is not, can in no way account for the phenomena of mutation which are common to both of these species. For this reason it seems important to describe the degree of mutability as it has been observed, up to this time, in *O. biennis*, which is, next to *O. Lamarckiana*, the most suitable species for this kind of research. The mutations in the other forms seem to be far more rare, and therefore require many more thousands of individuals for a statistical study or for experiments upon their causes.

Besides the assumption that *O. Lamarckiana* might be a hybrid, some authors have recently pointed out that hybridism may be one of the chief ways in which species are produced in nature, especially in the larger or so called polymorphous genera. LINNAEUS was the first to propose this hypothesis, at the time when the number of discovered forms was growing so fast as to make it almost impossible to assume a separate creation for every one of them. I have not the least doubt that LINNAEUS and his followers were right in this point, and that many wild species have been produced by the sexual combination of the characters of their allies. How great a rôle this kind of hybridization or of the recombination of char-

acters has played in the production of species in nature is a question which it is impossible to answer at the present time. There is no doubt that numerous hybrids are continually produced in nature, but almost all of them disappear after a relatively short period of existence. Even in such genera as *Cirsium* and *Salix*, which are known to be rich in hybrids, our knowledge concerning the propagation of hybrid strains is very small.[7] It is quite possible that some as yet undiscovered principle of purification (Selbstreinigung der Arten) prevails on a large scale, and if this should be so, we must expect hybrid races to be rather rare in the field.

FOCKE has published a list of forms which have been duplicated by means of artificial crosses,[8] and quite a number of later instances have been added to this list, the latest of them being the reconstruction of *O. biennis leptomeres* out of *O. biennis* L. and *O. atrovirens* Bartlett (*O. cruçiata* of my *Gruppenweise Artbildung*), by means of the expulsion of the undesirable characters in double reciprocal crosses.[9] But all such facts point rather to a relative rarity of hybrid races in nature, outside of the small number of well known polymorphic genera.

GATES assumes that crosses between species or between elementary species often occur in nature among allogamous or open-flowered forms.[10] But, according to my own experience, even in such cases hybrids are rare in the wild state, and hybrid races must be much rarer still. The slightest degree of weakening of the individual vigor will doom such hybrids to extermination, even as most of the occasional white flower mutations in nature disappear sooner or later, without starting a permanent variety.

In order to save the hypothesis of hybridism as a cause of the mutable condition of the evening primroses, different authors have

[7] For the hybrids of *Cirsium* see C. NÄGELI, Dispositio specierum generis *Cirsii* tam genuinarum quam hybridarum, in G. D. J. KOCH, Synopsis Florae Germanicae et Helveticae, pp. 743–760. 1857; and for the willows see MAX WICHURA, Die Bastardbefruchtung im Pflanzenreich, erläutert an den Bastarden der Weiden. Breslau. pp. 95, mit zwei Tafeln. 1865.

[8] FOCKE, W., Die Pflanzenmischlinge. 465–468. 1881.

[9] Gruppenweise Artbildung. Berlin. 311–312. 1913.

[10] GATES, R. R., Mutation in *Oenothera*. Amer. Nat. 45:577–606. 1911; see pp. 578–579.

proposed different auxiliary suppositions. And since the possibility is acknowledged that mutability may be far more widely spread within this group than we now know, such suppositions must not be of a limited nature, but applicable to large divisions of the vegetable kingdom. KEARNEY, in studying the mutations of the Egyptian cotton, comes to the conclusion that these and other mutations might be the result of crosses between remote ancestors, but that these crosses have left no other traces in their descendants than "the disturbance of germinal equilibrium, which manifests itself in the production of mutants."[11] It is not very clear how this supposition is to bring the problem nearer to its solution.

In a recent article in this journal,[12] JEFFREY takes an opposite position. He assumes that the ancestral crosses have left another visible trace in their descendants, which is the partial sterility of their sexual cells. It is a well known fact that many hybrids have partially sterile pollen, while acknowledged species have, as a rule, only fertile pollen grains. JEFFREY assumes this rule to be without exceptions, but does not adduce any arguments in favor of this hypothesis. It is difficult to judge the value of an argument so long as the facts upon which it rests have not been submitted to criticism. But I might suggest that it seems rather hard to reconcile this view with the fact that in angiosperms three of the four megaspores are usually sterile, while only one produces an embryo sac. Are we to deduce from this fact, in connection with JEFFREY'S hypothesis, that all angiosperms are hybrids, at least on the maternal side?

Numerous special arguments could be adduced. It may suffice, however, to point out the genus *Carex*, in some of the best species of which the pollen is in the same condition, three of the grains of each tetrad being sterile and only one fertile.[13] Every single grain of the ripe pollen is a tetrad, showing the very reduced rudimentary remnants of three of its cells as a flattened investment of the fertile one.

[11] KEARNEY, T. H., Mutation in Egyptian cotton. Jour. Agric. Research 2:287–302. 1914.

[12] JEFFREY, E. C., Spore conditions in hybrids and the mutation hypothesis of DEVRIES. BOT. GAZ. 58:322–336. 1914.

[13] JUEL, H. O., Die Entwickelung der Pollenkörner bei *Carex*. Jahrb. Wiss. Bot. 35:649–656. 1900.

In the article just quoted, no parallelism has been attempted between the presence of sterile pollen grains and the already numerous published instances of mutations outside of the group of the evening primroses. Let us take for instance *Capsella Bursa-pastoris*, which has produced *C. Heegeri* and *C. Viguieri.*[14] Its pollen is devoid of sterile grains. Here we have a clear case of partial sterility not being the cause of mutability. On JEFFREY'S principle we must acknowledge *C. Bursa-pastoris* as a good species of undoubted gametic purity, and therefore it is evident that even the purest species may be in a mutable condition. From this we infer that mutability in itself does not justify the supposition of a hybrid origin, and that attacks on the gametic purity of the evening primroses have no real support on this side of the question. I have cultivated both *C. Heegeri* and *C. Viguieri* in my experiment garden; the first of them has globular and the other four-winged capsules. Both are historically known to have arisen suddenly from the parent stock, and come true to seed.

JEFFREY lays stress mainly on the fact that partially sterile pollen is a widespread phenomenon among the allies of the evening primroses. Whether it runs parallel to their mutability has not been investigated, and as a matter of fact it does not seem to be much more highly developed in *O. Lamarckiana* and *O. biennis* than in the other members of the group.

The question of the partial sterility of the Onagraceae has been most thoroughly dealt with by GEERTS.[15] He sums up his results as follows: The genera *Jussieua*, *Zauschneria*, *Epilobium*, *Boisduvallia*, and *Lopezia* are wholly fertile; they show neither rudimentary ovules nor sterile pollen grains. Only in *Epilobium* and *Boisduvallia* some rare pollen tetrads may sometimes miscarry. In the genera *Clarkia*, *Eucharidium*, *Godetia*, and *Gaura* all the ovules are fertile, but among the pollen grains about 30 per cent

[14] SOLMS-LAUBACH, H., *Capsella Heegeri* Solms, eine neu entstandene Form der deutschen Flora. Bot. Zeit. 10:167–190. *pl.* 7. 1900.

BLARINGHEM, L., Fleurs prolifères du *Cardamine* des prés. Bull. Soc. Bot. France 60:304–311. 1913; and Les transformations brusques des êtres vivants. Bibl. Phil. Sci. Paris. 1911 (see pp. 119–147).

[15] GEERTS, J. M., Beiträge zur Kenntnis der Cytologie und der partiellen Sterilität von *Oenothera Lamarckiana*, Amsterdam. pp. 114, mit 24 Tafeln. 1901; see p. 93.

are sterile. *Kneiffia*, *Xylopleurum*, and *Lavauxia* have some rudimentary ovules as well as sterile pollen grains (10–50 per cent). In the genus *Oenothera*, with the subgenera *Onagra*, *Euoenothera*, and *Anogra*, the percentage of sterility is about 50 per cent in the ovary as well as in the anthers. In the first group about 40 species were studied, in the second 30, in the third 10, and in the last 40, making together about 120 species. If in the last three groups some species were pure, and devoid of sterile sexual cells, they would no doubt have been discovered, and the supposition that the remainder might be considered as their hybrids would have found support. But this was not the case, and if we wish to ascribe the presence of all these sterile sexual cells to ancestral crosses, the crosses must be supposed to have taken place, or at least to have begun, among the ancestors of the whole family, with the exception of the *Lopezieae*, the *Jussieueae*, and the *Epilobieae*. It seems hard to have to suppose that the whole pedigree of the *Xylopleurinae*, the *Clarkiinae*, and the *Oenotherinae* should have had to go through the development of partial sterility in order to produce the present mutability of *Oenothera Lamarckiana* and half a dozen or perhaps even a dozen of its nearest allies.

The second main supposition, namely that hybridism might be a cause of mutability, is dealt with by JEFFREY in a particular way. He assumes "that there is every reason to suppose that it has been an agency of great importance in *multiplying* species, although it is logically inconceivable in the present state of our biological knowledge that it could have presided at their origin." The first of these two alternatives represents, so far as I can see, a conviction which is at least very widely spread among biologists ever since the time of LINNAEUS. It by no means contradicts the theory of natural selection, nor that of mutation, nor any other evolutionary principle. It has no obvious reference to the phenomena observed in the evening primroses, since with them the production of new forms takes place in pure lines of a species which has come down to us unchanged during at least a century, since the time MICHAUX discovered it in the United States and sent it to Europe.[16] At least there is no direct recombination of characters

[16] The probable origin of *Oenothera Lamarckiana*. BOT. GAZ. 57:345–360. 1914; see *pl. 19*.

by actual crosses between different elementary types, such as we ordinarily suppose to occur in polymorphic groups in nature.

The other alternative, that it is logically inconceivable that hybridism could have presided at the *origin* of new species, coincides exactly with the current conception of the mutability in the evening primroses. New forms originate through the evolution of new characters, as in *O. gigas* and *O. rubricalyx;*[17] or through the loss of existing ones, as in *O. nanella* and *O. rubrinervis;* or by means of the appearance of qualities, which were probably latent in the parent race, as in *O. lata* and *O. scintillans*.[18] These cases are evidently not recombinations of existing characters. If it is conceded that the hypothesis of a hybrid origin does not apply to them, it is obviously unimportant for the theory whether or not, besides them, there are other instances which may be considered as hybrid recombinations. *O. semigigas*, which is a hybrid between a normal and a mutated sexual cell, has never been considered as an argument against the mutation theory.

In cultures of chrysomelid beetles, W. L. Tower has observed hereditary changes which run almost parallel to the mutations of *O. Lamarckiana*. He started from crosses between *Leptinotarsa decemlineata*, *L. multitaeniata*, and *L. oblongata*, and obtained constant races. When given proper treatment by changing their environic factors, these races could be made to break up, and they did so in a manner at least partially analogous to that of the evening primroses.[19]

It is obvious that the fact that mutations may be artificially induced in hybrid strains does not contradict the contention that they may arise in pure strains also. But from the experiments of Tower it seems that some hybrid strains at least are more liable to show the phenomenon.

[17] *O. gigas* is considered to be a progressive mutant on account of its double number of chromosomes and its special behavior in crosses. *O. rubricalyx*, which arose in the cultures of Gates from *rubrinervis*, and which I cultivated this summer from seeds kindly supplied by him, is perhaps the most beautiful among all the mutants of *O. Lamarckiana*. Its red color is something quite new in the group. It behaves as a Mendelian dominant in crosses with its parent species and is therefore obviously of a progressive nature; see Gates, R. R., Amer. Nat. 45:600. 1911.

[18] See Gruppenweise Artbildung. Berlin. pp. 244–260. 1913.

[19] Tower, W. L., Evolution of the chrysomelid beetles. Carnegie Institution of Washington Yearbook no. 12:68–71. *pl.* 3. 1913.

Let us now consider the production of new forms analogous to the mutations of *O. Lamarckiana* observed in allied species. The theoretical significance of these facts lies in the proof that any hypothesis to explain such phenomena on the ground of qualities which are special to LAMARCK'S evening primrose is to be considered as wholly inadequate.

The first instance of mutability shown by another species than *O. Lamarckiana* was the production of a dwarf by *O. biennis cruciata*, a form which is now to be described as *O. biennis* var. *leptomeres* Bartl. This form was first discovered in 1900 by my son ERNST DE VRIES in the sand dunes near Santpoort in Holland, where a single specimen of *O. biennis* bore linear petals, while all the surrounding individuals were normal *O. biennis* L. It had evidently arisen there by mutation.[20] From it a constant strain has been derived, which is still in cultivation.[21] Among about 600 plants of this variety a single dwarf arose in 1903.[22] It had all the marks of *O. biennis* L. combined with the stature of a dwarf and the linear petals of the parent form.

Shortly afterward STOMPS discovered, in his cultures of hybrids between this *cruciata* variety and the original species, another dwarf and, moreover, a new mutant type, *O. biennis semigigas*.[23] Both arose from guarded seeds without any intermediate steps, in the same way that the mutants of *O. Lamarckiana* are known to arise. They had cordate petals, the dwarf having in other respects the same characters as the dwarf of *O. biennis leptomeres*, and the *semigigas* having 21 chromosomes in its nuclei. STOMPS was the first to lay stress on these facts as a proof that mutability is not limited to *O. Lamarckiana*, and that, even if this latter species should have to be considered as a hybrid, mutability cannot be explained as a result of such a condition, since there is not the least doubt concerning the gametic purity of *O. biennis* L.[24]

[20] Die Mutationstheorie. Leipzig. 1900; see 2:599.

[21] Pure seeds of this pure strain I shall be glad to send to any botanist interested in these questions.

[22] Über die Dauer der Mutationsperiode bei *Oenothera Lamarckiana*. Ber. Deutsch. Bot. Gesells. 33:387. 1905.

[23] STOMPS, TH. J., Mutation bei *Oenothera biennis* L. Biol. Centralbl. 32:532. 1912.

[24] DAVIS, B. M., Mutations in *Oenothera biennis* L. Amer. Nat. 47:116. 1913; also Parallel mutations in *Oenothera biennis* L. Amer. Nat. 48:498–501. 1914.

From these discoveries it was pretty safe to deduce that the pure *O. biennis* must also be in a state of mutability, and the first thing to do was obviously to make extensive cultures in order to find the pure line mutants. STOMPS cultivated over 900 individuals of the third and fourth generations of a pure line, derived from a rosette collected by him in the sand dunes near Beverwyk, Holland, in 1905.[25] Among these he found one *O. biennis* mut. *nanella*, one *O. biennis* mut. *semigigas*, and also four instances of the pale-yellow variety *O. biennis sulfurea*. The first two he calls *parallel mutations*, since they are analogous to the dwarfs and *semigigas* mutations of *O. Lamarckiana* and arise in the same way and with the same differentiating characters. The experimental origin of *O. biennis sulfurea* by mutation clearly shows that this variety, which is anything but rare in some parts of our sand dunes, may arise in the same way in the wild condition and afterward propagate itself by seeds.

The production of dwarfs from *O. biennis* by mutation has since been repeated more than once in my cultures of hybrids between this species and some of its allies,[26] and a *lata* mutant from *O. biennis* has been reported by GATES and described under the name of *O. biennis* mut. *lata*. Besides *O. biennis*, some allied species also are now known to show the phenomenon of mutation. Among these, an American form of *O. biennis*, which I cultivate under the preliminary name of *O. biennis Chicago*, has been studied more extensively than any other form. I had already found in the neighborhood of Courtney, Miss., in 1904, in a locality called "the bottom," along the shores of the Missouri River, a single specimen with narrow, almost linear leaves. Evidently it constituted a wild mutation from the surrounding type.[27]

Seeds taken from the normal specimens of this locality have since produced in my garden two mutations, which proved, in their progeny, to give constant and uniform strains and which I have cultivated during a series of years under the names of

[25] STOMPS, TH. J., Parallele Mutationen bei *Oenothera biennis* L. Ber. Deutsch. Bot. Gesells. 32:179–188. 1914; also Parallel mutations in *Oenothera biennis* L. Amer. Nat. 48:494–497. 1914.

[26] Gruppenweise Artbildung. pp. 300–301. Berlin. 1913.

[27] *Op. cit.* p. 304.

O. salicastrum and *O. salicifolia*.[28] The first plants are as high as *O. biennis Chicago*, attaining 2 and sometimes (1914) almost 3 meters in height. They differ mainly in having narrower leaves. The *salicifolia*, on the contrary, is different from its parent species in almost all respects, being richly branched and rarely attaining one meter in height. It has almost linear leaves of a special blotted green, small erect flowers and long thin fruits. Analogous mutations have from time to time been observed in hybrid cultures of *O. biennis Chicago*.

Under the name of metaclinous hybrids I have described the curious phenomenon that heterogamous species from time to time produce among their hybrids from one cross, in one or a very few specimens, the type which is ordinarily that of the reciprocal hybrid.[29] For instance, the cross *O. biennis Chicago*×*O. Lamarckiana* gives the twin hybrids *densa* and *laxa*, while *O. Lamarckiana* ×*O. biennis Chicago* produces the twins *O. laeta* and *O. velutina*. Now among the first hybrid cultures sometimes a *velutina*, and more rarely a *laeta*, arises, and among the latter sometimes a *laxa*. Evidently some latent mutation, on the part of *O. biennis Chicago*, must be responsible for the production of these aberrant types. Analogous metaclinous hybrids have been described for *O. atrovirens* Bartl.[30]

Narrow-leaved mutations have also been seen in cultures of *O. muricata*, and of late (1914) in those of *O. suaveolens* Desf.[31] Moreover, *O. grandiflora*, collected by Mr. BARTLETT and myself at Castleberry in Alabama, throws off aberrant forms, one of which has broader and the other almost linear leaves.[32]

[28] For descriptions and figures see Gruppenweise Artbildung. pp. 304–307.

[29] *Op. cit.* p. 308.

[30] This is the species described in my book Gruppenweise Artbildung under the name of *O. cruciata*. For its metaclinous hybrids see pp. 309–310.

[31] For the different varieties and mutations of *O. muricata* see also GATES, R. R., A contribution to the knowledge of the mutating Oenotheras. Trans. Linn. Soc. II. Bot. **8**:1–66. *pls. 1–6*. 1912.

[32] For *O. grandiflora* see GATES, *op. cit.* p. 38. If the three types of *O. grandiflora*, observed in my garden, occur also at Dixie Landing, Alabama, and have crossed, each of them, with *O. Tracyi*, and have perhaps produced twin hybrids and unlike reciprocals, this might explain the large number of forms observed on that spot; see Science **38**:600. 1912.

Lastly, mutations have been observed by H. H. BARTLETT[33] to arise in *O. stenomeres*, a new species of Montgomery, Maryland. In the fourth generation of a pure strain, embracing 106 individuals, he found three aberrant types. One was a self-sterile plant, the second had thick buds and short thick fruits, and the third was a stout and very hairy individual with densely hairy petals, which justify its new name *O. stenomeres* mut. *lasiopetala*. Hairy petals constitute quite a new discontinuous variation among the evening primroses, since all individuals of *O. stenomeres*, as well as the allied species now being studied in this respect, have petals which are glabrous, except under microscopic examination.

From this list we see that at least seven species, besides *O. Lamarckiana*, are now known to be in a condition of mutability, namely *O. biennis* L., *O. biennis Chicago*, *O. muricata* L., *O. atrovirens* Bartl., *O. suaveolens* Desf., *O. grandiflora* Ait.,[34] and *O. stenomeres* Bartl. Probably more or less numerous allied forms will prove to be in the same condition as soon as they are tried on a sufficiently large scale. Therefore, this mutability can no longer be explained on the ground of observed or supposed characters of *O. Lamarckiana* which would distinguish this species from the other types of the group *Onagra*.

O. biennis L., the European type of the species, which is growing wild and in large quantities in the sand dunes of Holland, where it had already been observed and collected by LINNAEUS, is, next to *O. Lamarckiana*, the most suitable for researches concerning mutability. DAVIS says, "No wild species of evening primrose has been so long under experimental and field observation or is better known to the workers with Oenotheras than this plant. The species has proven uniform to a remarkable degree, and it would be difficult to find a type of *Oenothera* so free from suspicion of gametic purity. The species appears to have been in Holland since pre-Linnean days, and is therefore very old. As material

[33] BARTLETT, H. H., An account of the cruciate-flowered Oenotheras of the subgenus *Onagra*. Amer. Jour. Bot. **1**:226–243. *pls. 19–21*. 1914; see p. 236.

[34] Concerning the specific difference of the two last named forms, which have often been considered as synonyms, see L'*Oenothera grandiflora* de l'herbier de Lamarck, Travaux de biologie végétale dédiés à GASTON BONNIER, Rev. Gén. Bot. 25^2:151–166. *fig. 1*. 1914.

for experimental studies on mutation, the Dutch *biennis* seems to the writer the best of all Oenotheras so far brought into the experimental garden.[35]

In order to determine the coefficient of mutation for *O. biennis* L., I have made a culture of about 8500 individuals, all of which have been studied from their germination to the period of flowering and of fruiting. In the interest of subsequent cultures they have been pulled out before ripening their seeds, with the exception of a sufficient number of their mutants, which were cultivated with some of the true individuals in another garden.

The seeds for this culture were taken from the pure line pedigree plants of STOMPS, which were derived from a single rosette of radical leaves collected by him in 1905 in our sand dunes near Wyk aan Zee.[36] In this part of our country, no other species of *Oenothera* are growing and no intermingling of forms has to be feared. From seed of this plant, self-pollinated, a second generation was grown in 1910 and a third generation in 1912. Self-pollinated individuals of these two generations gave the seed for the cultures of STOMPS in 1913 and for mine in 1914. These latter came from three and four parent plants, the descendants of which numbered respectively 5500 and 3000. Of course I sowed almost all the available seed, and their culture just covered the field at my disposal outside of my experimental garden (about 600 square meters). Thus all my plants belonged to the same pure line as those of STOMPS, and the individuals which supplied the seeds had been cultivated under the most favorable conditions obtainable.

The seeds were sown in January, the seedlings transplanted into wooden boxes in March, and brought on the field in the middle of April. This early sowing and transplanting is with us the most effective means of making the plants annual, and in my whole culture less than a dozen individuals failed to flower.

It was possible, this time, to pick out the dwarfs from the wooden boxes before the transplanting into the field. By this means a second change of place was avoided, and the dwarfs could

[35] DAVIS, B. M., Parallel mutations in *Oenothera biennis* L. Amer. Nat. 48:499. 1914.

[36] STOMPS, TH. J., Parallele Mutationen bei *Oenothera biennis* L. Ber. Deutsch. Bot. Gesells. 32:179–188. 1914.

be cultivated together on a bed of my experiment garden, which enabled me to inspect them almost every day during their development and through the whole summer. The characters which distinguish the dwarfs in the stage of young rosettes, with leaves a few centimeters in length, were discovered in the following way.

The self-pollinated flowers of the dwarf specimen of STOMPS in 1913 had set no good seeds, but flowers pollinated from pure *biennis* had produced some fruits. Now my *O. Lamarckiana* mut. *nanella*, when crossed with *O. biennis*, yields only, or almost only, dwarfs. Therefore, the expectation was justified that such might also be the result of the cross *O. biennis* mut. *nanella*×*O. biennis*. Seeds from this cross had been sown about the same time; they yielded 108 seedlings, all of which have been planted out and have flowered. They were dwarfs without exception, reached in September a height of 40–45 cm. only, were richly branched, and had all the marks of *O. biennis* combined with the dwarfish stature and the liability to the same bacterial disease as is shown by the dwarfs of *O. Lamarckiana*. The young rosettes of these crossed *biennis* dwarfs clearly differed from the rosettes of the pure *biennis*. After the three or four first leaves with long petioles, there followed a group of leaves with smaller stalks and some sessile ones, thereby rendering the whole rosette far more compact than the corresponding ones of the pure *biennis*. With this character as a criterion, I isolated from my pure line boxes 8 individuals. One of them proved afterward to be a mistake; it was a pure *biennis*. Seven were dwarfs and have flowered; they were, in all external respects, like the crossed dwarfs of the control culture. Among the 8500 remaining plants I discovered later, in the field, only one dwarf. This shows that the characters were sufficiently reliable. All in all, I had 8 dwarfs in 8500 plants, making about 0.1 per cent. They occurred among the progeny of one of the self-pollinated mothers in the second generation (3 dwarfs), and of three of the parents in the third generation (5 dwarfs). Some of them have set good fruits after self-fertilization.

One of the most interesting and useful features of *O. biennis* L. is its propensity to make lateral rosettes from the base of the flowering stem. It is possible to isolate these rosettes and to have

them grow separately. The experiment succeeds easily if the rosettes have produced one or two roots of their own, however young and slender these may be. *O. biennis nanella* shows the same character, and in August I succeeded in isolating from my 8 pure line dwarfs 8 rosettes, all of which have since developed into healthy young plants with some long and narrow leaves, followed by almost sessile ones, quite different from the rosettes of normal *O. biennis*.

Moreover, two *nanella* mutants occurred in the cultures of *O. biennis sulfurea* which I shall have to describe later. These cultures were grown from self-pollinated seeds of the four *sulfurea* mutants of STOMPS (1913), and embraced over 1000 flowering individuals, the flowers of which were pale yellow without exception. Two of these plants proved to be dwarfs and were transplanted into my experimental garden. Both of them have flowered with pale flowers, have been self-pollinated, and yielded a sufficient harvest of seeds. The coefficient of mutation in this race was therefore 0.2 per cent, which does not differ essentially from the first instance (0.1 per cent). These dwarfs are the founders of a new race, *O. biennis sulfurea nanella*, which I propose to cultivate next year. Its pedigree name would be *O. biennis* mut. (1913) *sulfurea* mut. (1914) *nanella*. It is a double mutant, such as are quite common in horticulture, and shows the way in which wild species would have to be analyzed.

I used the pollen of the *O. biennis nanella* of STOMPS, in 1913, for two crosses, which may be briefly mentioned here. In the first place, I fertilized castrated flowers of the pure line of *O. biennis*. The pollen was not abundant, and I got only 15 good seeds, all of which have germinated and become stout flowering plants. They differed from normal *O. biennis* in no respect and at no moment during their development. Their self-pollinated seeds will have to be sown next year. In the second place, I pollinated *O. Lamarckiana* with the pollen of *O. biennis nanella*. From this cross I had a culture of 55 individuals, all of which have flowered. One of them proved to be a *lata* mutant, having besides the *lata* marks the same characters as its sisters. These were all alike and in no way different from the ordinary and well known type of *O. Lamarck-*

iana×*biennis*, which, moreover, flowered at the same time on other plots of my garden. A number of these plants have been self-pollinated. Thus we see that the pollen of *O. biennis nanella* gives in these cases exactly the same forms as that of pure *O. biennis*, at least so far as the first generation is concerned.

The specimen of *O. biennis semigigas* of the cultures of STOMPS had only matured seed in the capsules which had been pollinated by pure *O. biennis*, without being castrated. From these seeds two types arose, neither of which was a *semigigas*. All in all, there were 19 plants, belonging to two forms, besides a mutant. This last was a dwarf, which, however, has not flowered. Of the remainder, ten individuals were pure *biennis* during their whole life and in all their marks. They had the normal number of chromosomes, namely 14, and gave a normal harvest of seeds. The others, 8 in number, were different from these in almost all respects, though but slightly. The color of their foliage was a whitish green, the leaves more flat, and with white veins. The spikes were more elongated, the flower buds more slender, the flowers small and erect, the fruits thin and cylindrical and relatively poor in seeds. These plants had 15 chromosomes, like the *O. Lamarckiana lata* studied recently by GATES and Miss THOMAS.[37] But they had none of the characters of a *lata*, showing thereby that the number of chromosomes, even if differing from the type, does not necessarily run parallel with the external features.

Further studies will have to show why one-half of the progeny of this cross came true to the characters of the pollen parent, while the other half constituted a new and uniform type, differing from all the mutations and hybrids hitherto studied in my experiment garden; and especially why the characters of the mother of the cross should be wholly absent in its progeny.

The first result of this state of affairs has been that the characters which the *semigigas* mutants might show in early youth remained unknown, and that it has not been possible to point them out before the time of flowering. In July, all the spikes

[37] GATES, R. R., and THOMAS, N., A cytological study of *Oenothera* mut. *lata* and *O.* mut. *semilata* in relation to mutation. Quar. Jour. Micr. Sci. 59:523. 1914.

were carefully mustered and four specimens of the *semigigas* type were discovered. This makes a proportion of 4 to 8500, or about 0.05 per cent, showing the *semigigas* mutants to be only half as frequent as the *nanella*. On later inspections no additional cases were observed, and likewise intermediate or doubtful instances were absent. The four plants were exactly alike, save that three were very vigorous, and one, grown in a shady part of the garden, was very weak. The chromosomes were counted in the first three instances and found to be 21, as in the corresponding mutant of STOMPS.

My four mutants were easily discovered by their broad conical flower buds and their elongated spikes, which strongly contrasted with the dense spikes of the surrounding *biennis*. They reached the same height as these, the lowest flower being 90 cm. above the soil, and the total height about 1.5 meters. The leaves had the same form as those of *biennis*, but were a darker green and slightly more pubescent. The pollen consisted of 3- and 4-cornered grains, both of which types seemed fertile only for about a quarter. Artificial self-fertilization, however, had no result, and on the stigmas of *O. biennis*, *O. gigas*, and *O. Lamarckiana* the effect of the pollen was very slight, inducing some swelling of the ovaries but no good seeds or almost none. Inversely, I have tried to fertilize the flowers with the pollen of the three species named, but got a good result only in the case of *O. biennis*. Numerous good capsules with a sufficient supply of apparently good but in reality empty seeds have been obtained by leaving the flowers free to the agency of insects in the midst of the thousands of their flowering sisters, while in the same garden no other Oenotheras were grown.

The three vigorous specimens of the mutant produced some lateral rosettes at the base of their stem, even as we have seen in the case of the parent species and the dwarf variety. These rosettes were isolated and planted in pots in the beginning of August; four of them were very vigorous, but the other one rather weak. They have thrown off lateral rosettes themselves, and the stems repeated the production in two instances. It is proposed to try to bring these plants through the winter and repeat with them the culture and the experiments of this year. After a month, their

leaves reached 15 cm. and more in length and were clearly distinct from the normal type of *O. biennis*, being much broader and a darker green.

Of the four *semigigas* mutants, two arose from the seeds of the same parent which yielded the *semigigas* of STOMPS in 1913. All three belonged to the third generation of the pedigree. The two others were derived from two different parents of this same generation and therefore belonged to the fourth. The reason why three of the five came from the same lot of seed was probably no other than that the harvest of this plant had been the largest. More than one-third of my whole culture (3200 plants) were children of this mother.

No *gigas* with 28 chromosomes and fertile pollen occurred in my culture. With a chance of one sexual cell mutated into *O. gigas* in every 2000, the expectation for the copulation of two such cells is evidently only one in every 4,000,000. This would require a garden of more than five or six acres (two hectares) and the corresponding cost of labor. Perhaps some American institution is able to carry out the experiment. It may be reduced very essentially by a previous study of the marks of the young rosettes of *O. biennis semigigas*, so as to be able to plant out almost only these, hoping to find the *gigas* among them; or by studying the external influences which may increase the degree of mutability of the parents in the desired direction.

Sulfurea mutants have been far less rare. This was to be expected from the fact that STOMPS had 4 of them among 920 plants. From the parent type they differ only in the color of their petals, which is a very pale yellow. It is so pale that collectors, who see the variety in our sand dunes, often call the petals white. In the cultures they are easily seen as soon as the flowers open, especially in the evening. I found 27 of them among my 8500 plants, making a percentage of 0.3 per cent. They occurred in the progeny of all the 7 parents of my stock, 13 in the third, and 14 in the fourth generation. There were 6 parents, whose progeny contained 0.1–0.3 per cent, and one with 0.7 per cent (of the fourth generation). It is possible that this last parent had been more favored by external conditions than the three others of the

same group, although it grew among them and did not show any higher degree of vigor.

The fact that *sulfurea* mutants were observed in the progeny of every one of the 7 parents of my culture directly proves this line of mutability to be hereditary in the whole family derived from the 1905 rosette from Wyk aan Zee. In combination with the sporadic occurrence of the pale-colored variety in our sand dunes, we may further infer that this mutability is hereditary in the whole stock of our country, and probably also in the whole species, since *sulfurea* plants have been found from the time of TOURNEFORT in France and other European countries.

From the mutants constant races may be derived. I sowed the self-pollinated seeds of the four mutants of STOMPS, and cultivated 205, 225, 271, and 358 seedlings, altogether 1059 plants, all of which have flowered and produced only pale-yellow petals, making a very striking impression of constancy.[38] When crossed with the pure species, the *sulfurea* strains give uniform hybrids which are patroclinous. Those of *O. biennis*×*sulfurea* have the pale flowers, those of *O. biennis sulfurea*×*biennis* show the same bright yellow as the parent species.[39]

On experimental germination of seeds

Of the seeds of *Oenothera Lamarckiana* ordinarily only about one-third produce seedlings, and this proportion is highly variable, depending mainly on the conditions of cultivation of the parent plant. Among the remaining seeds some contain a normal embryo, others a more or less completely decayed one, while still others are empty. The last have been thoroughly studied by RENNER, who found that they have been fertilized as well as the normal seeds and those with decayed embryos. Between these normal and externally normal seeds are seen the numerous rudimentary ovules which have not been fertilized, and have not essentially increased their size after the fertilization of the others. These rudimentary seeds have been described by GEERTS, as referred to above.

[38] Self-pollinated seeds of this second generation of *O. biennis sulfurea* are available for exchange in return for other races of mutating primroses.

[39] Gruppenweise Artbildung. p. 298.

In the empty seeds the embryo develops only a little, just enough to stimulate the seed coats to an almost normal development, in size as well as in structure. For the most part these empty seeds are a little smaller and especially a little less broad than the others, and can therefore easily be picked out of a sample. But quite a good many are externally exactly like good seeds and cannot be distinguished from them without being opened. RENNER states that about one-half of the seeds are in this empty condition.

By means of a hard steel needle with a curved tip it is easy to make the seeds burst, especially after a thorough wetting. The seeds which contain a healthy embryo will discharge it; the unhealthy seeds will protrude a slightly brownish pulp; and the empty seeds show the lack of contents, except a thin layer of endosperm in the embryo sack. The various groups may be counted out in this way, but the limits between the originally empty seeds and those which have become more or less empty by an early decaying of their germs are not sharp and often dependent upon the health conditions of the seed-bearing individual.

Among the seeds with a normal and healthy embryo some will germinate during the first days after sowing, especially if the temperature is a favorable one. Others will follow sooner or later, some after weeks or months, while still others may remain dormant for years. It is not an uncommon case that the proportion of the rapidly germinating seeds is a very small one, and in this case a large quantity of seed is necessary to secure a small number of seedlings. Moreover, in those cases where the seeds do not produce a uniform progeny, but a mixture, as, for example, with twin hybrids or in hybrid splitting, the possibility cannot be denied that the numerical proportion of the components of the mixture may be different for the rapidly germinating seeds as compared with the others. In other words, percentage figures may be influenced to some degree by the occurrence of a more or less considerable proportion of dormant seeds.

In order to ascertain the value of this objection, I have made from time to time cultures in which the rapidly germinated seedlings were planted out separately from the slower ones. As a

matter of fact, I have not found as yet any essential differences between the two groups; but the doubt remained that such might still be discovered if it were possible to bring to germination all, or almost all, the slow seeds of a given sample. For a number of years I have tried various means to reach this end, but only of late have I succeeded.

It is a well known fact that many kinds of hard seeds may be induced to germinate by means of filing. Filing machines, especially for the smaller leguminous seeds, are now often used in agricultural practice, the best known one being the Swedish type, constructed by HJALMAR NILSSON, the Director of the Swedish Agricultural Experiment Station at Svalöf. It files the seeds in a continuous current by throwing them against a rapidly revolving filing disk. Unfortunately, in the seeds of the evening primroses, the hard layer is not the external tissue, but that of the inner integument. The outer coat thus prevents the filing, and experiments which Professor NILSSON has had the kindness to make for me with his apparatus did not give the desired result.

In the soil the water is imbibed into the seeds through microscopic and very narrow slits in the hard layer. It is assumed that these slits are filled with air which, in the narrower ones, is a powerful obstacle against the penetration of the water. So long as this only reaches the cuticularized parts of the walls of the slits, no moisture can reach the embryo and this remains dormant. The question, therefore, is to compel the water to penetrate into the deeper parts of the slits so as to reach the spots which can be moistened.

In order to solve this difficulty, I have tried pushing the water into the slits under a high pressure. A compression of the surrounding air to 6–8 atmospheres has proved to be sufficient to induce all or almost all the healthy seeds to germinate in a few days. The apparatus used is a combination of an autoclave with an air-pump such as is used for automobiles, and the model known as the Michelin pump seems to be the easiest and cheapest available one, while any autoclave, as, for example, an ordinary steam sterilizer, will answer the purpose. Mine has 20 cm. inside diameter, and can be filled to 8 atmospheres in about five minutes.

Before compressing the air in the seeds, these are thoroughly soaked with water. Ordinarily they are exposed in small tubes, half filled with water, to a temperature of about 30° C. during one night. In the autoclave they remain from one to three days, at the temperature of the room. My apparatus can accommodate over 100 tubes at a time, each containing a different sample. After leaving the reservoir, the seeds may be sown in seed pans for cultivation or may be left to germinate in the same tubes, if it is only intended to determine the proportion of seedlings produced. In order to do this the water is poured off through a small sieve, the tube is closed by means of a cork, and the seeds are distributed along the upper inner side of the tube, this lying horizontally. In this way they get exactly the required amount of water and of air for a vigorous germination.

I will now give some figures to show the effect of this pumping in of air into the previously soaked seeds. After pumping, the degree of germination was determined by leaving the tubes in a stove at 30° C. and counting the seedlings in samples of about 200 seeds each. Out of 18 capsules from self-fertilized flowers of a spike of *O. Lamarckiana*, 3400 seeds were counted, a separate germinating tube being used for the contents of each fruit. Of these seeds, 15 per cent germinated during the first two days and only 3 per cent during the two following days, showing the normal germination power to be almost exhausted. Then the seeds remained three days in water under a pressure of 8 atmospheres, after which they were brought back to the stove. The next two days produced 22 per cent seedlings, and the four following ones added only 1 per cent to this number. Then the remaining seeds were tried with a needle. Only about 5 per cent contained embryos, half of which at least were evidently in a decaying condition.

The total of germs was 46 per cent, leaving 54 per cent for those with an undeveloped germ. From these figures we see that the production of seedlings from a sample of seeds may be more than doubled by the pumping method, while all or almost all the healthy germs may be made to germinate. Numerous similar instances could be added.

A sample of seeds of *O. biennis*, taken from a late flowering individual, produced only 2 per cent of seedlings in the first two days, while a control sample, after having been exposed in water to a pressure of 6 atmospheres, produced at once 80 per cent of seedlings. In the same way for *O. suaveolens*, the percentage was increased from 3 to 14 per cent, for *O. muricata* from 12 to 80 per cent, and for *O. Cockerelli*, a species which is often very slow in germinating, from 2 to 72 per cent.

It is not improbable that in *O. Lamarckiana* the hard seeds may contain more mutants than the easily germinating ones, which have thus far been studied. It seems even possible that they may conceal some new, as yet unobserved, types of mutations. The new method enables us to bring almost all the germs to germination, as well as to separate the seedlings of the different groups.

Before concluding, I may be allowed to recommend this method for the study of various other kinds of seeds also.

Summary

1. In a culture of 8500 specimens of pure line *Oenothera biennis* L., 8 mut. *nanella*, 4 mut. *semigigas*, and 27 mut. *sulfurea* arose, giving the percentages of about 0.1 per cent, 0.05 per cent, and 0.3 per cent. In cultures of *O. Lamarckiana* the corresponding numbers are for *O. nanella* 1–2 per cent, for *O. semigigas* 0.3 per cent (Gruppenweise Artbildung. p. 329), while no color mutations have been observed as yet. With the origin of *O. Lamarckiana* the mutability for dwarfs, therefore, must have increased at least tenfold, and for *gigas* types about sixfold. The material cause for this improvement is in all probability the same as or closely connected with the cause of the largely increased number of mutative forms which are known to start from *O. Lamarckiana.*

2. From the cross *O. biennis* mut. *nanella*×*O. biennis* only dwarfs of a uniform type arose (108 Ex). *O. biennis*×*O. biennis* mut. *nanella* was in the first generation exactly like pure *biennis; O. Lamarckiana*×*O. biennis* mut. *nanella* exactly like *O. Lamarckiana* ×*biennis*.

O. biennis semigigas is self-sterile, but when pollinated by *O. biennis* gives for one-half pure *biennis* with 14 chromosomes, and for the other half a new, slender type with 15 chromosomes.

O. biennis mut. *sulfurea* easily yields constant races of a uniform sulphur color.

3. The question whether there is any causal relation between partial sterility of the sexual cells, hybridism, and mutability has to be studied in all those instances in which mutations are known to occur or to have occurred. In some of these cases, at least, the conditions are far more simple than for the evening primroses, as for example in *Capsella Bursa-pastoris*.

4. The mutative condition of *O. biennis* may be ascribed to some "germinal disturbance" of its hereditary qualities. Or, if we replace this vague and meaningless expression by a sharp hypothesis, we may assume as its cause the presence of one or more pangens in a labile position. The transition from *biennis* to *Lamarckiana* would then require the addition of one or more pangens in the same state, in order to explain the higher percentage of mutants and the larger number of their different forms. The presence of such labile pangens seems well proven by the results of numerous crosses.

The contention, however, that the transition of "undisturbed germinal material into a state of disturbance," or of one or more pangens from the stabile into the labile condition, may be induced by external influences in pure species, has not as yet found general acceptance. Some authors believe that crosses between different types are required to secure this effect. At this moment, it seems difficult to give experimental evidence for or against this view. Until this is reached, we must rely upon comparative studies in order to answer the main question whether or not the observed mutations in the evening primroses are analogous to those by which the mutation theory explains the evolution of the animal and vegetable kingdoms.

5. The mutants of *O. Lamarckiana* all agree with that species in certain characters, and not one of them shows any indication of a reversion toward any of the allied wild types. If the mutability was an effect of crossing, some marks, at least, of the other parent would be expected to reappear.

Besides this consideration, the available evidence lies in the fact that the derivatives of *O. Lamarckiana*, originated in my garden, differ from one another in marks, which are, although not

identical, strictly analogous to those which differentiate the wild species of the whole group. In some cases the differences are even larger. Those between the wild species are often very small and limited to certain life periods, leaving the species quite alike during the remainder of their development. No arguments have as yet been adduced to doubt the fundamental identity of the two groups of characters.

6. The phenomenon of mutability, observed in *O. Lamarckiana*, *O. biennis*, and allied forms, is therefore to be considered as a simple continuance of the supposed mutability which presided at the origin of the wild species of the evening primroses.

7. The seeds of the evening primroses are often very slow in germinating, leaving sometimes one half or more of the healthy germs in a dormant condition. This difficulty in the study of mutation percentages, etc., may be overcome by pressing the water into them. A pressure of 6–8 atmospheres during 1–3 days is ordinarily sufficient to stimulate all or almost all the good germs to a rapid germination.

The microscopic preparations and the counts of chromosomes, referred to in this article, have been made for me by my assistant Mr. C. Van Overeem, to whom I wish to give my sincere thanks for his cooperation.

Amsterdam, Holland

GROWTH STUDIES IN FOREST TREES

2. PINUS STROBUS L.

H. P. Brown

Object and scope of the investigation

(WITH PLATES XIII AND XIV AND TWO GRAPHS)

The present paper is the second of a series presenting the results of studies of growth in forest trees.[1] The investigations are planned with a twofold purpose, namely to clear up some disputed points regarding the formation of annual rings and to outline the laws of growth in trees. The results of the studies of *Pinus Strobus* L. are presented in this paper.

Pinus Strobus L. (white or Weymouth pine) was chosen for the second subject of investigation for several important reasons. First, it is a soft pine and differs from the hard pines, which include *Pinus rigida* Mill., both in external as well as internal anatomy. Further, it is more rapid in its growth than pitch pine, and interesting results were anticipated from a comparative study of the two trees. Finally, white pine is of recognized commercial importance, and it is hoped that some of the conclusions will prove of interest and value to foresters.

The specimens in the investigations, aside from the seedlings, were all in the wild state. The investigations were not limited to a few trees or to one locality. Specimens were examined in different woodlots, thereby providing variation in site, soil, and other silvicultural conditions. Seedlings from the nursery beds of the Department of Forestry, N.Y. State College of Agriculture, Cornell University, small trees from the same, as well as others in the wild state, and older trees which had passed the century mark and presented wide variation in crown development, were all subjected to examination. Fully 50 specimens were studied and from them comparative data were secured. It is hoped that the results thus obtained will prove of permanent value in formulating general

[1] The first paper appeared in Bot. Gaz. 54:386–403. 1912, and included the investigations upon *Pinus rigida* Mill.

rules of growth for the species. Inasmuch as a description of all the trees used would be confusing, short silvicultural notes on each specimen will be given in the text where it seems necessary.

Previous investigations of growth in forest trees

Before entering upon a description of the methods employed in this work, a brief résumé of those of other investigators will perhaps add interest to the present study.

VON MOHL (25) sought to determine the growth of trees by making measurements of the circumference at a definite place on the bole. From these the radius was computed and the increase in thickness noted. CHRISTISON (5) pursued the same method and computed data for a large number of species, including both hard and soft woods. The data of JOST (13) were based in part on the methods given above and in part on measurements which he obtained by the use of a "Fuhlhebel."[2] Any data secured through bark measurement are unreliable because of continual changes going on in the older parts of the secondary cortex and changes which bear no relation to the newly forming rings. As a result, only broad generalizations can be drawn from data based on such methods.

T. HARTIG (12) sought to determine the growth of tree species in a different manner. Choosing even-aged, pure stands, where growth conditions appeared to be similar, he felled typical specimens from these at different periods and made comparative studies. He assumed that in such stands all individuals exhibited similar characteristics of growth, a view that is untenable in the light of our present knowledge. HARTIG's method is open to criticism in that it was extremely inaccurate and could therefore never give reliable results. Growth varies markedly not only in different individuals in a stand, but also in different parts of the ring at a given height.

MISCHKE (24) took the first steps in the direction of securing accurate results. Using an increment borer, he studied the annual ring at different periods in its development and obtained in this way the first results which were in any way accurate. WIELER (39)

[2] For description see reference.

also followed this method and made many consecutive borings on coniferous and broad-leaved trees. His observations led him to infer that growth is very irregular not only as between different trees, but at different places in the same tree. The last named method, however, is subject to errors, and the results of WIELER show wherein it is inaccurate. When a boring is made with Pressler's increment borer, it is impossible to avoid applying some pressure to the wood core which is to be removed. During rapid growth (fig. 4) the elements of the newly formed xylem are thin-walled and easily crushed and displaced by pressure, however slight. The partially formed ring when treated in this manner may easily show a wide variability in diameter and thus lead to grossly erroneous results. This appears in part to explain why WIELER inferred that in neighboring areas growth varies considerably. I have already pointed out (1) that slight differences occur in neighboring areas, and the present investigation leads to the same conclusion, but the marked discrepancies in growth which WIELER describes are not present in either of the pines which have been examined.

Histological technique

The methods pursued were in general those followed in 1910. The technique, however, has been improved as the time and place to secure the best material became more evident with increasing experience. The chief objection is that it is necessary to make rather large wounds on the trees. This objection is not so serious, however, in coniferous trees because the resin which exudes prevents quite efficiently the drying out of the tissues.

The investigations on white pine began in August 1912, and continued until October 1913. Incisions were made on trees at intervals from base to crown (as high as it was safe to go in tall trees). Unless otherwise stated, these were always on the south side of the tree. A few cuttings were made on the north side for comparative purposes. Cuttings from branches at intervals were also made, and, unless stated otherwise, were lateral on the branch. Each cutting included all or a portion of the inner bark, the cambium, and all of the preceding year's ring except toward the end

of the season, when the growth of the years had attained such thickness as to make this impracticable. Duplicate cuttings were made on several trees at intervals of time varying from a few days to several weeks. These were always near the former cuttings and lateral to them. Rarely were the duplicate cuttings more than a few inches from the original ones. One series began in August 1912 and continued into September of the following year, cuttings being taken at least every month.

The material, as soon as obtained, was properly labeled and fixed in Gilson's fixer of the usual strength. Then after thorough washing it was run up through the lower grades of alcohol and stored in 65 per cent alcohol until wanted. The above corresponds to the methods used in 1910.

From this point the procedure varied depending on the object in view. When it was desirable to know the extent to which growth had progressed, or the abundance of starchy material present, freehand sections were made with a sharp razor. These were dyed with temporary stains and then studied. The HCl-phloroglucin stain for lignin, followed by the permanent Haidenhain's iron-hematoxylin stain for cellulose, gave excellent results. With this combination the extent of growth could be measured and estimated with accuracy. Chlor-zinc-iodide, as well as various combinations of I-KI, were occasionally used.

For minute examination of the tissues greater care was taken in manipulation. The material was demineralized with hydrofluoric acid and imbedded in celloidin. Sections were obtained in this manner 10-15 μ thin, which served the purposes of the investigation. Extensive and comparative histological studies were then made and the results tabulated.

The method described above makes it possible to follow the progress of the growth. One obstacle which could not be overcome was the inaccessibility of all parts of standing trees. In order to check up the results obtained, several trees of different ages were felled during the growing season. Accurate stem analyses were made of these and the progress of growth was observed throughout the tree. These data were then compared with those obtained from standing trees.

Investigations on the roots of trees were attempted and gave some results worth noting. The roots were secured from young trees (about 30 years). Other root studies were made on seedlings from the nursery. A few cuttings were taken from the roots of old trees where they were exposed near the base of the bole.

Microscopical characters of the xylem

The xylem of white pine is so well known that it is unnecessary to describe it here. When contrasted with the wood of pitch pine, the xylem of white pine differs in a number of anatomical features. The upper and lower walls of the ray tracheids are smooth as compared with the dentate ones of *Pinus rigida*. Bordered pits occur on the tangential walls of the late wood, while they are lacking apparently in pitch pine. The transition between early and late wood is not so abrupt in white pine and the rings are generally wider. White pine is a more thrifty tree, and the present observations tend to show that it is more susceptible to changes of site, soil, etc., than pitch pine. This is exceedingly important from the economic standpoint.

The roots of white pine exhibit the usual features of the roots of Abietineae. Diarch, triarch, and tetrarch roots are common. The root of seedlings is usually diarch (fig. 8), but the number of xylem rays is as a rule soon increased to three or four.[3] Vigorous roots from thrifty trees between 15 and 30 years of age were prevailingly tetrarch, so that it would appear as if the number of xylem rays is correlated in some way with the amount of moisture available to the root and to the root environment, whether favorable or otherwise. VAN TIEGHEM (34) has noted this same variability in *Pinus*, *Abies*, and other allied genera, and further has pointed out that no constant relation prevails between the number of xylem rays and the number of cotyledons. Not only does the number of xylem rays vary in roots of different ages, but the number may increase or decrease during the growing season. This is strong evidence that environmental conditions influence within

[3] Each xylem ray is terminated centrifugally by a resin canal, and the oligarchy of young roots can in this way be easily determined in cross-section with the naked eye.

certain limits the number of xylem rays. In general the larger the diameter of a yearling root, the greater the number of rays up to four. A pentarch or hexarch arrangement was not observed in any case, although it may occur, since VAN TIEGHEM (*op. cit.* p. 7) reports the number as high as 7 in Scotch pine (*P. silvestris*).

The xylem in old roots is comparable to that in the aerial parts of the tree, but differs in several well known particulars. The tracheids in roots have wider lumina and thinner walls and are never as well lignified as those in the parts above ground. This is especially well seen in cross-sections. In roots late wood formation is not as pronounced, owing no doubt to a decrease in mechanical strain in underground parts. The bordered pits on the tracheid walls, in both roots and stems, are mainly radially arranged. The uniseriate arrangement is here and there interrupted by the pairing of some pits. Further, the bordered pits in roots are larger than in the xylem of aerial parts. It is of interest to note in this connection that wherever an old root becomes exposed it usually presents xylem typical of aerial parts, so that only underground parts exhibit the characteristics above described.[4]

Winter condition of secondary cortex and cambium[5]

The secondary cortex of white pine is very similar to that of pitch pine. It presents the same radial arrangement of the elements, this arrangement becoming less regular as they are pushed to the outside (fig. 1). Companion cells are totally lacking, but one distinct row and a few scattered bast parenchyma cells are formed each year as in pitch pine, and these indicate the annual phloem areas in the old cortex. Occasionally the phloem parenchyma becomes crystallogenous, but never attains the size of that of pitch pine. The marked differences which exist between the bark of white pine and pitch pine are not present in the young phloem, but are caused by changes which take place subsequently in the outer cortex.

[4] KNY (20) has pointed out the same structure in *P. silvestris*, and found that it was especially pronounced on the underside of large roots which had been exposed through erosion.

[5] The notes only include observations on the winter condition of aerial parts, as underground parts were not accessible at this time of the year.

The resting cambium as seen in cross-section consists of 2–10 layers of tabular cells lying just outside the last formed xylem elements. SANIO and other early anatomists considered the cambium as consisting of a single initial layer, which through repeated division gave off daughter cells centripetally and centrifugally. These divided a second time, and the resulting cells enlarged and became elements of the phloem or xylem. The initial layer, if present in white pine, cannot be distinguished as such. The cells in each layer of the merismatic tissue are similar to the others in size, form, and protoplasmic content. The number of layers of cells in the cambium apparently varies between rather wide limits. Such variability might lead one to think that the work was not accurately done. The figures given above are as exact as the material permitted. The cambium is a very variable tissue both in number of cell layers and thickness of the same. Data indicating this (table A) were computed from measurements on tree I, a description of which follows.

Tree I was a thrifty specimen of white pine standing near the edge of a mixed stand on the brow of an incline which sloped northward into Six Mile Creek valley. The height of the tree was 55 feet, and crown development extended to within 18 feet of the ground. The tree was directly exposed to the south. Ground cover included sparse brush and pronounced sod formation. Cuttings were first made on this tree in August 1912, at heights of 4, 17, 30, and 43 feet from the ground, and were repeated at frequent intervals until September 1913, as recorded in the tables. Twig cuttings from near the top of the crown were also taken from time to time. The data in the table are from the cuttings of February 20, 1913.

TABLE A

WINTER CONDITION OF CAMBIUM, TREE I

Cutting	Thickness of cambium	Cell rows in cambium	Average width of last formed rings
2 year twig........	5– 9 μ	2– 4	691.0 μ
6 year twig........	5– 9	2– 4	661.0
12 ft. from apex.....	29–31	6– 7	3367.0
25 ft. from apex.....	32–40	7– 9	4297.6
38 ft. from apex.....	29–35	7–10	3013.8
51 ft. from apex.....	29–35	7– 9	2894.0

It follows from the table that in white pine trees which are growing rapidly, the cambium is smallest both in number of cells and thickness in the smaller twigs and branches. It increases gradually in thickness and number of cell layers until that point is reached in the bole where diameter growth is a maximum. The decrease in the figures indicating the dimensions of the cambium are not proportional thereafter with the decrease in growth in diameter. It would appear as if the cambial layer, once it had attained its largest proportions, varied little in vigorous trees. In suppressed trees, however, it may reasonably be assumed that the cambial layers fall off in number and thickness toward the base of the shaft, but in such cases the reduction is not closely correlated with decrease in the width of the completed annual ring.

Another point relating to the cambial and phloem tissues deserves description here. I refer to the statement commonly made in textbooks that while the formation of xylem ceases early, the cambium continues to form phloem as long as climatic conditions are favorable. It is of interest to note in this connection the condition of the young phloem and cambium on September 26, 1912, and February 22, 1913. In all four cuttings of the first named date we find the condition as shown in fig. 7. Xylem formation had apparently ceased, the cell walls in the last row of tracheids were still in the process of thickening, but no new elements were being added. In the phloem we find a broad band of sieve tubes with a few parenchyma cells interspersed among them, making up in all some 15 rows of cells. This represents, with the possible addition of two or three rows of partly crushed elements to the outside, the seasonal growth of phloem. It is to be noted here that none or very little compression had occurred.

Comparing the above with what occurred on February 20, 1913 (figs. 2 and 3), the following interesting changes are to be found. Contraction had taken place, due to low temperatures during the winter, but not all of the sieve tubes are flattened to the same extent. In each of the four cuttings of February 22, the 3–5 last formed sieve tubes are only partially distorted by pressure, and those in the higher cutting (fig. 2) noticeably more so than in the lower cutting (fig. 3). In the last case there is a sharp dividing

line between the compressed sieve tubes and those which exhibit no compression. Further, as will be shown subsequently, the latter are the first sieve tubes to function the following spring. We may say that in white pine phloem development continues longer than xylem development. It only ceases with the extreme cold temperatures of December and January, and the tree makes no special provision for cessation of growth as in the xylem. Sieve tubes in all stages of arrested development may be found during the dormant period.

General discussion of tree growth

Growth as it occurs in trees falls logically into two subdivisions: growth in length and growth in thickness. In the first category we have only primary growth. It does not matter whether elongation is going on in root, stem, or leaf, it always has its inception in a growing point, and all tissues resulting from cell divisions in this apical meristem are primary tissues. Growth in thickness, on the other hand, results mainly from secondary thickening which is brought about through the activities of a perennial cambium. Tissues arising in this way are distinguished as secondary tissues in contrast to primary tissues.

The primary tissues, with the exception of the primary cortex, usually soon attain their full size in both coniferous and dicotyledonous trees, and in the majority of woody plants we may regard them, with the one exception mentioned, as mature at the end of the first growing season.[6] Secondary growth, however, commonly begins the first year, and as a result the processes of primary and secondary thickening overlap, and both often go on at the same time in closely neighboring parts of the tree. Secondary thickening may thus occasion alterations before all the primary tissues have reached the adult state. It is entirely conceivable, for example, that both categories of growth go on simultaneously in the terminal shoot of a pine or in a young root. In this connection URSPRUNG (35) reports in certain cases the subsequent enlargement of the pith after secondary thickening had begun, so without

[6] It is only in woody monocotyledons and tree ferns that primary growth persists for any length of time.

doubt these two processes overlap. For the sake of clearness in the subsequent discussion, however, growth in length will be considered in the general sense of primary growth, growth in thickness as secondary growth.

Awakening of secondary growth in aerial parts[7]

The awakening of secondary growth in aerial parts is first manifested in the cambium and the adjacent phloem tissue. It is evident in all cases several weeks before actual cell division begins. The cambial cells and last formed sieve tubes (6–10) open out radially, so that they attain several times their former diameter. Reference to the following table will show the changes which occurred between February 22, 1913 and March 29, 1913, in the width of cambium and last formed phloem.

TABLE B

GROWTH WITHOUT CELL DIVISION, TREE I

Cutting	February 22, 1913	March 29, 1913	Per cent increase	April 12, 1913	Per cent increase
I..........	69.0 μ	91.5 μ	32.6	013.3 μ	49.7
II..........	89.1	110.6	24.0	182.9	105.3
III..........	94.1	140.7	70.0	180.4	81.0
IV..........	103.3	171.1	56.0	188.5	82.0

In all cases, enlargement of the tissues in question occurred between the first two dates, and amounted from over a quarter to nearly three-quarters of their original size. The place of greatest enlargement was in cutting III, 17 feet from the ground. This does not correspond with the place of greatest ring thickening (table A) the previous year. Whether any significance can be attached to this discrepancy cannot be decided with certainty. It seems reasonable, however, to ascribe it to heightened temperature from the rise of soil water in the xylem.[8] It would be natural to assume

[7] No observations have been made on secondary growth in the leaves. MEISSNER (21) has observed a marked increase in the number of phloem elements and a very slight increase in the xylem elements in a number of species of *Pinus*.

[8] ROBT. HARTIG (11, p. 262) made note of the temperature of soil water as a factor potent in forwarding growth.

that the greatest increase would be in cutting II. However, as this was 30 feet above the ground, it is quite possible that the tissues there had not yet experienced the increase in temperature due to the rise of soil water in the trunk.

The present investigation gives no reliable data as to where the first phloem activity was manifest. It had occurred throughout the tree on March 29, 1913. The awakening of growth began in this one specimen before the first of April and was not accompanied by cell division. Soil water was apparently largely instrumental in its inception.

If we refer again to table B for the data for April 12, 1913, two weeks later, we may draw the following interesting conclusions. The greatest diameter increase at this time is in cutting II, where it has been over 100 per cent. In other words, the ascending soil water may have reached the point of greatest growth (because the previous year's ring was widest here) and caused a rapid expansion of the tissues. In cuttings III and IV we find an apparent reversal of the foregoing conditions. Cutting III has increased only 11 per cent during the same period, while in cutting IV we find the increase has been 26 per cent. This may be ascribed to two causes, either one or both of which may be responsible. While the increased temperatures may have prevailed longer in IV than in III, the amount of reserve food material available was not as great. As a result, growth in cutting IV may have been retarded more than it was in cutting III; or cell division may have occurred in some of the cuttings and upset the equilibrium. Careful counts were made to find the number of cells in the cambium and last formed phloem in all four cuttings of March 29 and April 12. While slight differences occurred, these were not such as to warrant the conclusion that cell division had taken place between the two dates. The changes which occurred between March 29 and April 12 were due solely to enlargement of cells already present. We must infer then that the apparent contradictions of the figures in table B are due to differences of available food in different parts of the tree.

Cell division had begun in tree I by April 26. At this time the activity was manifest in cutting I (table C). Here some 8–12 tracheids and 2 or 3 new sieve tubes were already formed. Wall

thickening had not begun in the new tracheids, but was noticeable in the first of the new sieve tubes. Cutting II exhibited only slight evidences of cell division, while in cutting III growth was well started. No division had yet occurred in cutting IV. Growth had been very rapid in cuttings I and III, as evinced by the absence of thickening of the cell wall. This may be accounted for in part by the high temperatures which prevailed between April 22 and 26. During that period the mean daily temperatures ranged from 52° to 70° F. Precipitation amounted to only 0.03 inch, but large amounts of ground water were available at that season.

TABLE C

BEGINNING OF GROWTH BY CELL DIVISION, TREE I, APRIL 26, 1913

Cutting	Growth	Number of tracheids	Wall thickening	New sieve tubes
I........................	Present	8–12	None	2–3
II........................	Indication	0– 1	None	0–1
III........................	Present	6– 9	None	1–2(?)
IV........................	None	0	None	0

To explain plausibly the conditions in cutting II, the point of greatest growth the preceding season (table H), is not an easy task. Every indication seems to show that we might expect most rapid growth at this point. We can conclude only that the restricted growth here denotes one of the many idiosyncrasies of tree growth, where, as pointed out by WIELER (38), marked differences may occur in closely neighboring spots. It is to be expected that growth would not be manifest in cutting IV at this early date, so we may conclude that in tree I cell division was in evidence on April 26 in the upper portion of the bole but had not yet begun at the base.

In order to check the results on tree I, four bole cuttings, including the terminal leader and four branch cuttings, were made on a neighboring tree on May 4, 1913, eight days later. Tree II stood about 10 feet from tree I, and was apparently of the same age and subject to the same silvicultural conditions. The extent of growth and lignification in this individual is given in table D.

It follows from table D that on May 4 cell division was going on in all parts of the bole on the south side of the tree, with the exception of the terminal leader. It was farthest advanced in cuttings II and III, as might be expected, because the last formed annual ring was thicker here than in the basal cutting.

TABLE D

EXTENT OF GROWTH IN TREE II, MAY 4, 1913

Cutting	Number of tracheids	Amount of lignification	Remarks
I. Terminal leader, 1 year's growth	None	None	Phloem active; no cell division
I. Same, 2 years' growth	1– 2	None	Same as above
II. 44 feet above ground	12–15	1–3 tracheids	New phloem elements. cell division
III. 33 feet above ground	12–15	1–2 tracheids	Same as above
IV. 5 feet above ground	10–12	None	Same as above

The branch cuttings were made on a branch which ran out some 20 feet in a southeasterly direction at a distance of 13 feet above the ground. Five cuttings were taken at intervals of about 4 feet. In cutting I (beginning from the tip) no tracheid formation was evident. In cuttings II and III tracheid formation was in progress, while in IV and V (basal) enlargement of the tissues had occurred, but no cell division. Cell division in the branches was similar to that in the bole, but more sluggish. It begins back of the branch leader and is most tardy in the base and leader of the branch. Which of the last is the first to exhibit growth depends on the length and vigor of the branch.

Numerous other observations were made on trees of different ages and different localities. It was found that cell division may be in progress some time in the upper portions of a tree while it is totally lacking below. This applies especially in old mature trees in closed stands, where growth is proceeding very slowly. Further, in general cell division first begins on the south side of the tree and in the basal portions. This peculiarity, due apparently to insolation, has also been observed in some cases in pitch pine (1).

It was noted in reviewing the literature on tree growth, that some (14) have attempted to correlate growth awakening with

the opening of the buds and the formation of new leaves. With this point in view, measurements were made on a young white pine stand of natural origin on May 3, 1913. The trees varied in age from 4 to 11 years and were in a thrifty, vigorous condition. On this date the buds were found to have opened and the young stems to have elongated 0.5–2.5 inches. All the growth in length had occurred in the preceding 10 days. Neighboring trees which were older showed less pronounced elongation of young parts; but growth had been in evidence in older, less favored trees since March 29, 1913, and cell division at least since April 20 of the same year. It follows, therefore, that growth in thickness begins before growth in length, and apparently, at the start at least, at the cost of reserve food material. No correlation exists between the two in white pine.

Concerning the time of cambial awakening, the researches of others bear out the conclusions of this paper. Some of CHRISTISON'S (5) are given in table E. It should be noted, however, that CHRISTISON'S results were obtained from bark measurements and do not necessarily indicate xylem formation.

TABLE E

GROWTH AWAKENING IN CONIFEROUS SPECIES, EDINBURGH, SCOTLAND

No. of tree	Name	Year	Awakening of growth
8	Abies Lowiana	1890	April 6
92	Abies Lowiana	1888	April 16–30
66	Abies Douglasii	1890	April 20
6	Abies Douglasii	1888	April 16–30
91	Abies	1890	April 13
2	Pinus excelsa	1890	April 6
26	Pinus Pinaster	1890	May 3
39	Cedrus africana	1888	April 16–30

MISCHKE (24) also made observations on this point, but did not include white pine among the species investigated. WIELER (39), however, examined three white pines in his experiments of 1894. Two of these were from a closed 40 year old stand, the third a 15 year old tree from another stand, all near Dresden, Germany. Growth was in evidence in the younger specimen to the extent of 13 or 14 tracheids on April 24. No growth occurred at the base of

the others until after the first of May, but growth must have been in evidence in the higher portions of the tree before that date. In the latitude of Dresden growth apparently starts fully as early as at Ithaca.

Intensity of growth in aerial parts

As already noted, growth continues some time before cell division occurs. It is first manifest through the enlargement of tissues already formed during the previous year. When cell division begins, it proceeds at first very rapidly, and in such a way that more elements are added to the inside of the cambium than to the outside. This was plainly observed in the sections and included in the data in table C. There 8–12 tracheids have been formed, as compared with 2 or 3 new sieve tubes. The cells thrown off to the outside gradually become transformed into sieve tubes, or more rarely into phloem parenchyma cells. This is accomplished in the first case through a thickening of the cell wall and the formation of lateral sieve plates. The phloem parenchyma cells thicken their walls very little at first, but enlarge for several seasons and eventually attain a much larger size than the sieve tubes. In the outer bark their walls are often strongly lignified.

Evidence of the rapidity of xylem formation is readily obtainable in early May. It is not uncommon to find 10–15 tracheids fully formed (fig. 5) without any indication of thickening of the wall. Subsequently the thickening begins, and before it has progressed to any extent lignification is evident in the cell walls, as brought out by the phloroglucin-HCl reaction. Wall thickening and lignification never start, however, until tracheids have attained their maximum dimensions as seen in cross-section.

The rapidity of vernal growth in white pine is apparently contingent on three factors: (*a*) the amount of reserve food material, (*b*) moisture, and (*c*) temperature. The first is always at its optimum in the spring, as the abundance of starch in the storage tissues testifies. Moisture likewise, at this time of the year, is available long before the buds begin to open. GOFF (8) has pointed out the early resumption of growth in the roots of coniferous plants, and observations on white pine coincide with his results. The

first two factors, therefore, may be eliminated from the discussion because both are at an optimum in the spring.

The temperature of the cambial layer depends, on the other hand, on three factors: (*a*) temperature of the air, (*b*) temperature of the soil water, and (*c*) direct insolation. Which of the three is most potent in the awakening and rapidity of cell division has not been determined, because they are so closely related to each other. It would appear that the temperature of the soil water plays a prominent part in the awakening of growth because of the opening out of the phloem first near the base of the tree. Factors (*a*) and (*c*) would be entirely negligible here, or at least play a minor part because of the thick layers of bark. Growth in the spring begins before factors (*a*) and (*c*) could have reached any appreciable height, so that the heat derived from soil water is certainly potential in awakening growth.

It is quite impossible to separate factors (*a*) and (*c*) and to note their effect in all trees. However, cuttings secured from the north and south sides of isolated trees at the same height often afford ample evidence of the effect of insolation.[9] Data were secured to bear out the foregoing statement as early as May 10, 1913. The tree examined was a "Wolf" white pine on the south side of Fall Creek beyond Forest Home, N.Y., a suburb of Ithaca. The specimen was 51 feet high, with a diameter breast height of 15.3 inches, and exhibited vigorous growth in spite of the poor soil conditions. At the date mentioned, tracheid formation had proceeded on the south side to the extent of 12–14 tracheids, while on the north side 9 or 10 tracheids had been formed. Lignification had not as yet set in, although all but the last 3 or 4 tracheids formed had apparently attained their ultimate size. That direct insolation is potent in the awakening of growth in trees is certain. However, one individual will present occasionally conditions the reverse of what would be expected.

It follows from the preceding paragraph that the awakening and the rapidity of growth depends on three factors, two of which are at an optimum in the spring and may therefore be neglected.

[9] Trees should be selected only from sites which are level, as trees growing on slopes are subjected to other factors which often overshadow the effect of insolation.

The third (temperature) is a variable one, and to this the rapidity of cell division is apparently directly proportional. Some idea of the rapidity with which the formation of tracheids may go on may be drawn from the following. Basal cuttings were taken from the south side of the "Wolf" tree already described on May 3 and May 10, 1913. The first cutting showed no evidence of formation of tracheids, while the other, taken a week later, exhibited 7 tracheids in each row, complete as to size, with several smaller ones in process of formation. The growth in places farther up the stem must have been going on still more rapidly. While the period mentioned above was warm and humid and therefore especially conducive to rapid growth, it may be safely assumed that in all white pine trees in the vicinity of Ithaca formation of tracheids is very rapid at the start. A large number may be formed in a relatively short time.

Intensity of growth in aerial parts

In the discussion which follows, the distinction between intensity of growth and amount of growth must be kept clearly in mind. The latter may be easily ascertained for the whole growing season or for any part thereof by measuring at a given period the amount of new tissue. Growth intensity, on the other hand, is constantly changing. It may vary from week to week, day to day, and even within one and the same day, as FRIEDRICH has pointed out (**7**). The amount of growth during a given period is then the sum of the prevailing growth intensities multiplied by the time each was in force. Let us take a specific example. Suppose a white pine first begins the formation of new xylem on May 1, and, on May 30, 60 new tracheids were in evidence in each tracheid row. It does not follow that 20 tracheids were formed the first 10 days, and 20 during each succeeding 10 days, making a total of 60. While the average growth intensity was two tracheids per day, the actual growth intensity may have vacillated on either side of this amount. It is obvious that it is quite impossible through comparative studies to obtain the prevailing growth intensity at a certain definite time. In order to do this the growth process would have to be actually observed. Some idea of the variability in growth intensity may be

gained, however, by comparing the average growth intensities for different periods in the growing season. It follows that the shorter the intervening time periods, the greater would be the vacillations in the data. With this idea in view table F was constructed. It includes figures of growth intensity from tree I during the summer of 1913. The width in microns of the new growth is given for 6 different periods, together with the total increase from period to period and the average gain per day. The last will give, not the actual, but the average intensity of growth of the period just preceding.

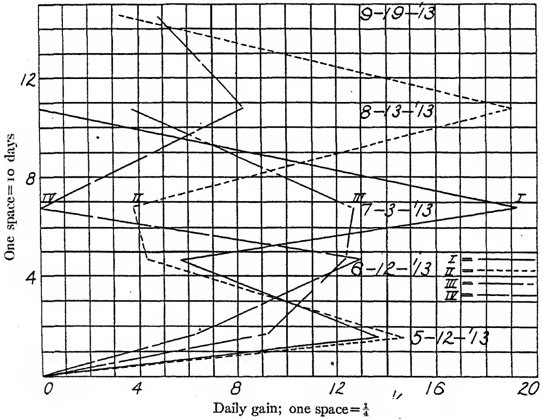
If the data of April 26 and May 12 are compared, the following points are to be observed. The average growth intensity was greatest in cutting II, with cuttings I, III, and IV following in the order named. On June 12, 31 days later, cutting IV exhibited the greatest average growth intensity, with cuttings III, I, and II following in the order named. Again, in the cuttings of August 18 and September 19, different combinations occur. At the first named date, growth was more rapid in cutting II, while at the latter date it was in cutting IV.

TABLE F

GROWTH AMOUNT AND INTENSITY, TREE I; SEASON 1913

No.	Amount 4–26–'13	No. of days	Gain	Gain per day	Amount 5–12–'13	No. of days	Gain	Gain per day
1......	170.0 μ	....	....	....	716.3 μ	16	546.3 μ	34.1 μ
2......	26.6	....	....	....	609.6	16	583.0	36.5
3......	106.7	....	....	....	472.4	16	365.7	22.9
4......	0.0	....	....	....	243.8	16	243.8	15.2
	6–12–'13				7–3–'13			
1......	1164.2 μ	31	447.9 μ	14.4 μ	2176.0 μ	21	1011.8 μ	48.2 μ
2......	1021.1	31	411.5	13.3	1224.0	21	202.9	9.7
3......	1414.3	31	941.9	30.4	2067.2	21	652.9	31.9
4......	1251.2	31	1007.4	32.5	1219.2	21	....	....
	8–13–'13				9–19–'13			
1......	2176.0 μ	41	0.0 μ	0.0 μ	2176.0 μ	37	0.0 μ	0.0 μ
2......	3100.8	41	1876.8	47.5	3394.6	37	293.8	7.94
3......	2448.0	41	380.8	9.3	2339.2	37	....	....
4......	2067.2	41	848.0	20.7	2502.4	37	435.2	11.8

The results given in table F are represented in graph 1. The abscissas indicate the daily gain and the ordinates the time intervening in 10 day periods. From the way in which the lines cross and recross, it is evident that average growth intensity and the actual amount of growth which is correlated with it vary between wide limits during different periods. The cambium may be very active for a time, then slacken in its growth, this to be followed again by renewed activity, with a final slump toward the end of



Graph 1.—Curves of growth intensity, tree I, 1913

the growing season. All the cuttings represent these two fluctuations except cutting III, and this departure may be accounted for through the inequalities of growth in closely neighboring parts.

Two periodic optimums of growth intensity have already been noted by others. Friedrich (7) made observations with the help of calipers, and found that in both coniferous and hardwood trees there were two periods of growth, one lasting until the end of May, then sinking some until the middle of June, followed later

by another maximum again in July, and then rapidly diminishing and ceasing altogether. JOST (13) has carried on observations which substantiate those of FRIEDRICH. It is remarkable how well the deductions of these two investigations are brought out in graph 1.

The first optimum is without doubt made at the expense of the reserve food supply. It is not until June and even later that the bulk of the seasonal results of metabolism in the leaves is available. This causes the second optimum, which may occur in July or August. It might be said in this connection that the amount of moisture and the prevailing temperature has been responsible for the results in table G. The meteorological data which follow

TABLE G

METEOROLOGICAL DATA, SEASON 1913

Month	Mean temperature	Precip. in 0.01 in.	Month	Mean temperature	Precip. in 0.01 in.
April	48.1	1.49	July	70.4	1.59
May	55.4	3.15	August	69.6	1.92
June	65.0	2.00	September	61.0	3.28

are the best refutation of that argument. The decline occurred in three cuttings between the middle of May and the third of July, yet the temperatures prevailing were not such as to warrant this, nor was there a noticeable decline in the precipitation. My observations agree with those of FRIEDRICH, that there are in white pine at least two periods of maximum growth.

Irregularity of secondary growth in aerial parts

A thorough treatment of the increase in growth in trees must necessarily be very comprehensive. The study in all its phases is a comparative one, for only by resorting to comparison can any fundamental rules of tree growth be formulated. A comprehensive study should therefore treat comparatively of the growth of (*a*) one individual during one growing season, (*b*) of one individual from season to season, (*c*) of different individuals in the same stand during one season, and finally (*d*) of different individuals not in the same stand. Data bearing on the first, second, and last phases of the

subject are available. For the third the reader is referred to the publications of WIELER (37–39), JOST (13–15), R. AND T. HARTIG (9–12), MISCHKE (24), and others.

The amount of seasonal growth in an individual or of growth up to a given point in the season is equal to the sum of the products of the different prevailing growth intensities by the time each was in operation. It follows that these summations would be quite different in different parts of the tree. The only reliable method to indicate the amount of seasonal growth at a given point and at a given time is as a percentage of the previous year's ring. Even this is open to criticism, in that the annual increment often varies between wide limits from year to year. Yet general deductions may be drawn from data of this kind which will indicate to some extent at least the amount of growth at definite times. Table H was made with this idea in view. The figures were obtained from tree I. The width of the previous year's ring represents the average of the last formed rings as exhibited in the 6 different cuttings.[10]

TABLE H

AMOUNT OF GROWTH IN PERCENTAGE OF PREVIOUS YEAR'S RING, TREE I

No.	4–26–'13	5–12–'13	6–12–'13	7–3–'13	8–13–'13	9–19–'13	Average width preceding ring
I..........	3.8	16.0	26.0	48.7	....	....	4471.7 μ
II.........	0.7	17.2	28.9	34.6	87.7	96.0	3535.3
III........	4.8	18.0	50.2	78.9	93.5	89.3	2619.4
IV.........	0.0	11.8	60.4	58.8	99.7	....	2071.7

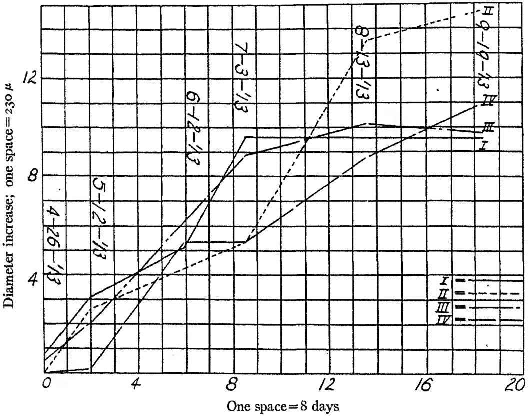
On April 26 the greatest proportion of new growth occurred in cutting III, with cuttings I, II, and IV following in the order named. On May 12 the order remained the same except that cuttings I and II had interchanged. On June 12 the ring was, theoretically at least, from one-fourth to over one-half complete; on July 3 from one-third to over two-thirds complete; while on August 13 seasonal growth was over four-fifths complete in all cases.[11] After the last named date, the growth was very sluggish

[10] The last acts as a control and tends to eliminate error.

[11] Growth ceased in cutting I after July 3, 1913; see table F.

for the next 37 days. Though still in evidence at the end of that time, we may infer that the ring was to all purposes complete.

The contents of table H are presented in graph 2, where the abscissas represent the diameter increase of new growth and the ordinates 8 day periods. It is to be seen from both the table and the curves that in the long run the cambium in all parts of the tree tends to even up irregularities which arise from different growth intensities. If growth is sluggish at one place in the bole and rapid



GRAPH 2.—Curves of growth amount, tree I, 1913

in another during a given period, the pendulum of growth intensity swings to the other extreme in the next period and evens up the disparity.

The irregularities of growth are manifested not only in the actual dimensions of the new formed tissues, but in the number of new xylem elements as well. If careful average counts of new formed tracheids are made, and these cômpared with the average number of tracheids at that point in the preceding ring, irregularities

crop out all along the bole of the tree. The data given in table I illustrate this very well. They were computed from tree III on June 17, 1913. This tree was a vigorous specimen on the north end of a small island in Fall Creek, east of Forest Home. The height was 81 feet, the diameter breast height 21.5 inches,[12] and the age about 80 years. Exposure was to the southeast. Crown development was good but one-sided, and greatest to the southeast. Root development was also uneven, but the reverse of crown development, because the constant washing of the water and the mechanical action of ice had destroyed the root system on the east side. The tree was felled on June 17, 1913, and a double series of

TABLE I

Extent of growth in tree III, June 17, 1913

Cutting	N.W. side		Percentage	S.E. side		Percentage
	Tracheids in old ring	Tracheids in new ring		Tracheids in old ring	Tracheids in new ring	
I	55–65	20–25	0.38	55–65	20–25	0.38
II	32–34	15–18	0.52	30–35	15–17	0.48
III	22–24	9–11	0.43	30	18–20	0.63
IV	23–25	10–12	0.46	22	17	0.77
V	16–18	6–8	0.41	19–21	13–15	0.70
VI	16–18	6–7	0.35	23–25	14–15	0.63
VII	15–16	7–10	0.60	12–14	9–10	0.69
VIII	12–14	7–9	0.61	9–12	6–8	0.64
IX	12–14	5–7	0.46	12–14	7–9	0.62

9 cuttings taken at intervals of 10 feet, one on the northwest side and one on the southeast side (exposed). Careful average counts were then made of the number of new formed tracheids, as well as those in the preceding ring at each point. On June 17, 1913, growth was more advanced in every cutting on the southeast side, the first two cuttings excepted, than on the northwest side. This was to be expected, because of direct insolation and larger crown to the southeast. But in both series of cuttings marked vacillations in growth are evident, so that it follows that growth irregularities express themselves not only in a variability in thickness of tissue, but also in the number of elements laid down.

[12] Exceptionally high because of the buttressed base.

Growth irregularities in individuals have been noted by others, although their results are in some cases to be questioned because they were based on external measurements alone. Such are those of CHRISTISON (5), VON MOHL (25), and JOST (13); reference to whose work has already been made. The results of TH. HARTIG (12) and ROBT. HARTIG (10) can have no significance in this connection, inasmuch as individuals were felled to secure data and consecutive measurements were quite impossible. MISCHKE (24), as already noted, employed an increment borer, and his results, with those of WIELER (39) who pursued the same method, are more reliable though not as accurate as is desirable. The former made comparative notes on Norway spruce and Scotch pine, and his results clearly indicate growth fluctuations. WIELER subjected

TABLE J

GROWTH OF WHITE PINE AT DRESDEN, GERMANY

Date 1894	I		II		III	
	Ring breadth mm.	No. of tracheids	Ring breadth mm.	No. of tracheids	Ring breadth mm.	No. of tracheids
April 24	....	....	....	....	0.48	13–14
May 5	....	....	....	....	1.12	26
May 16	0.08	3–4	0.14	8–10	2.34	47
May 26	Lost	Several	....	....	2.00	47–50
June 5	0.11	3–4	0.18–0.20	4–6	4.12	89
June 16	0.06	2–4	0.12	6–9	3.15	72
June 26	0.15	6–8	0.16	6–8	2.74	73
July 7	0.19	8–9	0.46	15	5.61	113
July 17	0.45	15–17	0.47	13–16	6.67	150
July 28	0.23	8–11	0.53	20–23	9.23	175
August 7	0.42	17–18	0.32	15	6.25	150
August 18	0.34	12–18	0.31	14	5.68	135
August 28	....	15–20	0.30	13–15	5.22	119
September 8	0.26	11–13	0.55	23	9.32	218
October 17	0.38	13–19	0.38	16	....	....
November 1	0.26	11–13	0.49	20–22	9.26	212

more trees to the same inquiry, and his investigations are of greater interest because he worked on white pine. Table J indicates his results on the three different specimens mentioned previously, and in each case fluctuations in growth are marked. The work of BUCKHOUT (2) serves to accentuate the same point. He made bark measurements on a white pine and a larch which extended over a

period of 4 years. The results were plotted in curves where the abscissas represented 5 day periods and the ordinates increase in circumference in sixteenths of an inch. In each of the four curves for white pine, from several to many growth fluctuations are evident. Still other researches could be cited to emphasize the same point. Many irregularities in growth occur during the season of cambial activity.

Comparative growth studies between different individuals of white pine (not in the same stand) were also carried on during the summer of 1913. In such investigations only temporary mounts were made and the necessary data secured from these. A few extracts from this part of the work follow.

On May 10 two cuttings were made at the base of the "Wolf" tree previously described, one on the north side and one on the south side. The first exhibited about 10 tracheids (7 complete) as to size on this date, while 12–14 tracheids were in evidence on the south side. On May 9 two cuttings were secured from a large white pine which presented different conditions of site, although in the same vicinity. This was a mature specimen some 110 feet high and 22 inches diameter breast height. It stood in a mixed hardwood stand where the land sloped sharply to the south. Ground cover was sparse. The tree, while mature, appeared to be still in vigorous growth. The north cutting (next to the bank) exhibited 2 tracheids, neither complete as to size; while in the south cutting no new tracheids were to be seen. Without doubt growth was going on vigorously in the upper part of the tree at this date. Observations on the same tree at a later date showed similar discrepancies. On June 13 the south basal cutting of the "Wolf" tree showed 20–25 tracheids already formed; 15 or 16 of these had apparently attained their ultimate size. The same cutting from the older specimen at that date possessed 11–13 new tracheids, 7–9 of which had attained approximately their maximum size. The amount of growth was decidedly less in the older individual.

Even wider discrepancies may be expected than the above where the differences in age are greater. For example, a young tree was examined on the same date (June 13). This was a thrifty

14 year old individual situated in the midst of the stand and only a few feet from the "Wolf" tree. In fact, the "Wolf" tree may have been one of the seed trees from which the stand had arisen. The cutting was made at approximately breast height, and already on June 13 the annual ring exhibited some 90 new tracheids. Three weeks later summer wood formation began. It follows that up to July 1, at least, we may expect many discrepancies in growth to occur. The greater the difference in vigor between the two trees compared, the greater will be the difference in the amount of growth at that period.

Others have noted the same growth irregularities between different individuals of the same species. Among these is ROBT. HARTIG (10), who expresses himself emphatically on this point. I quote from his text as follows:

> Bei freiem Stande und directer Insolation des Baumes, besonders aber des unbedeckten Bodens beginnt der Zuwachs in den unteren Stammtheilen weit früher, als im geschlossenen Bestande und bei einem Boden, der entweder beschattet (Nadelholzstand) oder von einer dichten Humusdecke bekleidet ist. An 100 jährigen Fichten, welche isolirt an einem Südhange standen, war schon am 1. Mai auf Brusthöhe der Dickenzuwachs in Thätigkeit, an ebenfalls frei stehenden gleich starken Bäumen des Nordhanges auf nasskaltem Boden war am 26. Mai noch kein Zuwachs bemerkbar. Im vollen Waldesschlusse zeigten manche Fichten und Kiefern selbst am 1. Juni noch ruhendes Cambium auf Brusthöhe, u.s.w.

An excellent illustration is likewise afforded by WIELER's table (table I). Trees I and II were in a 40 year stand, where they had been subjected to similar silvicultural conditions. WIELER failed to say whether tree II was bored on the north or south side, but in either case the tracheid numbers are seen to be quite different from those in tree I. Growth curves from neighboring trees under similar conditions never coincide. Fluctuations are constantly arising which upset the regularity of growth and for which no one factor is responsible.

Termination of secondary growth in aerial growth

The autumn condition of the cambium was observed in tree I both in 1912 and 1913, inasmuch as cuttings first began on this tree on August 5, 1912. The data given in table K include the

results obtained from two cuttings in 1912 and the last two cuttings of 1913. The table is of value because it offers comparative data which are strongly correlated with the results of others. While the periods of time between the cuttings of 1912 and 1913 are different, it is obvious that in each year the greatest increase of xylem toward the end of the growing season was in the basal cutting. In other words, growth continued vigorously at the base of the shaft until well into September, while in the higher parts it had either

TABLE K

TERMINATION OF GROWTH, TREE I; 1912 AND 1913

Cutting	Width of ring		Amount of increase	Percentage of increase	No. of days	Percentage of daily increase
	Aug. 5, 1912	Sept. 26, 1912				
I..........	2643.8 μ	3176.9 μ	533.1 μ	20	52	0.38
II..........	3046.4	....	....	....	52	....
III..........	2529.6	2622.1	92.5	3.6	52	0.07
IV..........	1550.4	2328.3	777.9	50	52	0.96
	Aug. 13, 1913	Sept. 13, 1913				
I..........	2176.0 μ	2176.0 μ	....	....	31	....
II..........	3100.8	3394.6	293.8 μ	9.5	31	0.31
III..........	2448.0	2339.2	....	....	31	....
IV..........	2067.2	2502.4	435.2	21.1	31	0.68

totally ceased, as in cutting I, 1913, or continued very sluggishly,[13] and this condition was exhibited by tree I during two successive years.

It follows from the preceding paragraph that in normal white pine trees growth is apparently first retarded above, retaining its vigor longest in the basal portions of the bole. The results of others on coniferous species lead to the same general conclusion. T. HARTIG (12) worked on both hard and soft wood trees and came to

[13] The disparity in the data of cutting I for the two consecutive years may be questioned. In 1912 there was an apparent gain of 20 per cent during the period intervening between the two dates given, while in 1913 no growth was evident at all after the first date. But in 1912, the first cutting was made on August 5, while the following year it was 8 days later. This probably accounts for the increase in the first case. Growth was still in evidence there on August 5, but had the cutting been made 8 days later, the results might have been decidedly different.

the conclusion that cessation of growth occurred later below and last of all in underground parts. R. HARTIG (11), following up these studies, made cuttings from species of *Pinus*, *Picea*, *Larix*, and *Abies*, in order to determine the condition of the cambium in different parts of the shaft. Cambial activity in each case was farther advanced above than below. It gradually diminished in intensity during the months of May, June, and July in the higher parts, while below the same applied to the months of June, July, and August. KNUDSON'S data (16) indicate the same condition of the tissue for *Larix laricina*, except that in the larch the phenomenon occurred in July instead of August and September.

The disparity in growth in different parts of a tree is without doubt dependent on conditions of temperature. The primary cortex persists in white pine for a long period, in some cases as long as 50 years. This condition is brought about through the division of the original cells of the cortex by anticlinal walls, and the subsequent enlargement of the two cells thus resulting. Meanwhile, cork formation remains superficial, so that the upper portions of the tree, even where the bole is 15 inches in diameter, are clothed by a layer of living, chlorophyll-bearing, primary cortex. Sooner or later, however, and varying markedly in different individuals, deep cork formation begins. This is evident first through the formation of isolated areas of brown tissue which stand out sharply from the surrounding living cortex. These increase in number, finally become confluent, and the characteristic old bark of white pine is formed. With this change in the type of cork formation there is correlated a modification of at least one factor potent in forwarding growth. The first phellogen is continuous around the whole circumference and functions until deep cork formation begins. New cork cells are added to the outside, and with the increase in circumference the older ones on the extreme outside slough off. So long as the primary cortex persists, the corky mantle remains thin and its protective value is in like proportion restricted. With deep cork formation, however, the conditions are altered to a large extent because the corky layers which are then formed through the activity of each phellogen accumulate. Protection of the cambium in the basal portions of the tree is thus greatly increased.

Changes in temperature are less effective there because the thick corky layers tend to equalize the conditions which prevail at different times during the growing season. Cool autumn nights, for example, would chill the cambium in the upper parts of the tree much earlier than below. Temperature changes become operative first where the primary cortex still persists, that is, where the bark is yet smooth. This without doubt explains the disparity of growth as we find it in white pine. Growth is first retarded above, but may go on vigorously below for a much longer period.

The exact time of growth cessation apparently varies widely in different species, in different localities, and in different sites. While wide variations occur, still certain generalizations apply. At the outset the term "growth" is a misnomer. As already noted, phloem formation, at least in conifers, does not cease with xylem formation, but continues uninterruptedly until late in the fall. It is necessary, therefore, to discuss xylem and phloem separately in their relation to cessation of growth.

A comparison of cuttings from tree I for the years 1912 and 1913 will give an idea of the seasonal termination of xylem formation. One discrepancy was noted at the start. In spite of the fact that the final cuttings in 1912 were a week later than those in the following year, growth was apparently more vigorous at the later date in 1912 in all four cuttings. This is to be explained in two ways. It was due either to seasonal variations or to the fact that the vigor of the tree had been materially lessened the second year through the many cuttings taken from it. An examination of the meteorological data for the two seasons has added no convincing evidence, inasmuch as comparative figures of growth for the two years were not at hand for a sufficiently large number of individuals, and general assumptions were therefore out of the question. Possibly both factors were in force.

To give the exact time or a definite place in the tree for the termination of xylem formation is quite impossible, as the data on tree I indicate (table F). In 1912 growth was still in evidence on September 26 in all four cuttings, as transitional forms of tracheids could be noted in every case (fig. 6). Growth, however, was going on at this date very sluggishly. Often only one flattened transitional

tracheid occurred between a cambial cell and a fully formed (as to size) tracheid, and occasionally here and there in a cutting this was lacking entirely. Again, in the cuttings of September 19 of the following year the same condition of affairs existed. While growth as to relative amount had to all purposes ceased, still all indications pointed to the fact that in all four cuttings it was going on, though very slowly. In both cases growth appeared to be most sluggish in cutting III, but no reason can be assigned to account for this fact.

The data from the preceding paragraph lead to the following conclusions. Xylem formation in tree I continued during two successive years until the last half of September, possibly even as late as October 1. Further, it was in evidence throughout a large part of the bole, as cutting I was 38 feet above the ground and the terminal leader extended only 17 feet beyond. Whether it still continued in the terminal leader cannot be concluded from the present investigation. If we correlate these deductions with those previously reached in the paper, the following points are evident: (*a*) growth intensity falls off first in the upper parts of normal white pine trees, more tardily below; (*b*) cessation of xylem formation does not follow the same law, but persists sluggishly in all parts of the bole (with the possible exception of the terminal leader) until the latter part of September; (*c*) the exact time of the end of xylem formation was not determined in the present investigation, but it is safe to conclude that it was practically complete by October 1.

The results of the present study are contrary to those of ROBT. HARTIG (9), who says, "Der Abschluss der Zuwachsthatigkeiter-folgt oben entsprechend früher, als unten." Too much emphasis must not be placed upon this statement, because (1) HARTIG made external measurements only, and (2) his results may have been influenced by subsequent phloem formation after xylem formation had ceased. ROBT. HARTIG avoids the issue in part when he states that cambial activity occurs in the tops of trees in *Pinus silvestris*, *Picea excelsa*, and *Larix decidua* during the months of May, June, and July, and at the base during June, July, and August. While he implies also a cessation of growth, he does not say it in so many

words. WIELER (39) has given some data concerning the termination of growth from the three white pines which he investigated (table J). In tree I the ring was complete on the north and south sides at the base on September 8. In tree II it was complete on the south side at the same height on August 28, while in tree III it was still in progress on September 8. In general his results indicate that in the vicinity of Dresden, Germany, growth in white pine ceases slightly earlier than at Ithaca, N.Y., a reasonable conclusion, since the former is in a higher latitude. The work of BUCKHOUT (2), already cited, is of interest in this connection. While his measurements were made externally and are therefore subject to the same criticisms as those of TH. HARTIG, certain facts are obvious. During the four years over which his experiments extended, growth was manifest in the white pine during the last 10 days in August and in two as late as September 8. His results serve to accentuate the fact that white pine has a long growing season, much longer than the European larch, with which he also worked.

The growing season of tree I may be used, in spite of variations which occur between individuals in that respect, as a general indicator of white pine growth in the vicinity of Ithaca. As already indicated, growth in white pine may be divided into two periods: (A) growth without cell division and (B) growth with cell division. B of necessity follows A. Considering A and B together, growth began in tree I before March 29, 1913, and continued until after September 19 of the same year, a period of over 5.5 months; and this does not include the late phloem development which without doubt continued into October. Cell division began before April 26 of the year in question, and if growth is considered in the narrow sense, the period is shorter by several weeks. If there are any grounds for the statement that trees complete their seasonal growth in a period of 4 or 5 weeks, white pine is an exception to the rule, as here the growing season extends over a period of 4–5 months, depending on the interpretation of the term "growth."

Differentiation in the annual ring in aerial parts

In working up the foregoing data, no stress has been laid on differentiation within one and the same annual ring. As is generally

known, each normal ring may be divided into spring wood and summer wood, or better early wood and late wood. The second of these two regions is distinguished from the first either by a diminution in the size of the vessels, as in the case of ring porous woods, or through a reduction in size and flattening of the elements formed in the outer part of the ring. The proportion of early and late wood in the ring affects strongly the physical properties of the wood, and as a result the early workers gave much time to its consideration. The factors controlling the amount as well as the time of late wood formation have been a subject of inquiry, and a hasty review of the literature on the subject, as well as a summary treatment of the results of this study from this viewpoint, are appropriate here.

One of the first theories offered to account for the variation in ring was that of KRAUS (19), SACHS (30), and DEVRIES (36), who explained it through differences in bark pressure at different times during the growing season. The radial pressure was at a minimum in the spring, permitting a greater expansion of the new elements, while it gradually increased during the growing season, ending with a maximum. The pressure leading up to the last was responsible for late wood formation. This theory was disproved by KRABBE (17, 18) beyond all contention in 1882, and since that time a number of new theories have sprung into existence, each with adherents.

ROBT. HARTIG (9) sought to explain the late wood formation in that the cambium was but poorly nourished in the spring. Late wood formation depended upon improvement in the nutritive conditions later in the season. According to HARTIG the size of the lumina of tracheids is dependent on the amount of transpiration of the foliage, while the thickening of cell walls is correlated with the increased amounts of food available at that time of the year. Diametrically opposed to HARTIG'S theory is that of WIELER (37) and RUSSOW (29), which was based on the assumption that the early wood owed its origin to better conditions of nourishment.

STRASBURGER (32) accepts neither of these theories, but explains annual ring formation as a normal fixed process. The young wood is the response, according to his theory, on the part of the plant

to a demand for conducting tissue, while the late wood is formed to increase the stability of the tree. The last factor may have been in force from the beginning, but was at the start overshadowed by the first.

MER's theory (23) rests firmly on the general assumption of WIELER as given above. According to his idea, the early wood results when the cambial activity is at a maximum, that is, in the spring, while late wood formation occurs when growth is going on very sluggishly, as in August and September in the white pine. The last elements of the annual ring are flattened because with the falling off of growth intensity the radial stretching of the young elements subsides in the same proportion.

Still another theory is of interest here because it departs decidedly from all of those mentioned. SCHWARZ (31) assigns the chief rôle in late wood formation to longitudinal pressure. This is in force throughout the growing season, but its effects are lost at first as the result of other factors, such as nourishment, which are temporarily more potent at that time. With a decline in the action of these, the effect of longitudinal pressure (gravity) reasserts itself.

No attempt has been made in the present work to refute or substantiate any of the theories above mentioned, nor in fact to bring forward a new hypothesis for annual ring formation. Other workers of the last decade have given the matter serious thought, but the problem still remains unsolved. It is the opinion of the author that several factors are potential, but inasmuch as these cannot be controlled by the investigator, the precise influence of each on growth cannot be definitely determined. The results obtained appear to substantiate MER's theory to some extent, in that growth in tree I was more rapid in the spring and early summer than subsequently. But the assumption that the cambium was better nourished at the beginning of growth than later is not justified from the present inquiry. It can only be said, in conclusion, that late wood formation occurs at a time when growth is proceeding very slowly.

No definite results were obtained concerning the time that late wood formation begins. White pine does not lend itself to a study

of this sort, because the transition from early to late wood is always very gradual, and it is difficult to distinguish the first formation of late wood tracheids. Larch should prove much more satisfactory for this study. But in spite of the difficulties mentioned above, it was obvious that late wood formation was in evidence in tree I on August 5, 1912, and on August 13 of the following year. On each of these dates all four cuttings showed some traces of it, and, further, it appeared to be slightly more advanced in cuttings I and II than in the ones taken lower on the bole. This was to be expected from what has been previously said; late wood formation begins first in the upper portions of the bole.

Primary growth in aerial parts

Some attempt was made in the investigations to secure reliable data concerning the elongation of aerial parts. A sample plot of 0.05 acre was laid off on May 3, 1913, in the vigorous young white pine stand mentioned previously. It included 115 trees which ranged from 4 to 11 years. The soil was of medium thickness, underlaid by sandstone and shale; exposure was open. All the trees were seemingly vigorous.

The trees were first examined on May 3 as to elongation of aerial parts. At that date elongation had already begun in all the trees on the plot, which varied from 0.5 to 2.5 inches. Greatest elongation had occurred in the terminal leader, while it was less pronounced in the slower growing lateral branches. The leaf fascicles were in evidence, but had as yet attained no appreciable length.

Observations corresponding to the above were subsequently made on May 30, June 17, and July 4 of the same season. Accurate measurements of the terminal leader and of the preceding year's growth for 50 trees were made in each case and the results collected in table L. The average growth of the preceding season is considered as the mean of the average preceding year's growth as found on May 30, June 17, and July 4. It is to be observed that the last vary slightly, as no attempt was made to select the same 50 trees on each date. The measurements are given in inches and fractions of inches.

Elongation of aerial parts began in the young growth in question before May 3 and continued until about July 4. Assuming that growth before May 3 proceeded at the same rate as between May 3 and May 30 (1.2 per cent a day), we can only infer that the awakening of growth in length in the shoots began about 8 days before (April 25), a conclusion that field observation fully confirmed. Cessation of growth in length in shoots had occurred by July 4, without doubt because at this date the length of the season's growth had surpassed that of the preceding year, and furthermore the terminal cluster of buds was fully formed. It follows from the data that in 50 young trees in 1913 elongation of the terminal shoots

TABLE L

GROWTH IN LENGTH IN THE TERMINAL LEADER

Date	Average seasonal growth	Average growth of preceding season	Mean growth of preceding season	Per cent of preceding seasonal growth	No. of days	Per cent gain	Per cent gain per day
5- 3-1913....	1.25	....	13.55	9.3	......	......	......
5-30-1913....	5.6	13.98	13.55	41.3	27	32.0	1.2
6-17-1913....	10.02	13.22	13.55	73.9	18	32.6	1.8
7- 4-1913....	14.37	13.45	13.55	106.1	17	32.2	1.9

began in the last part of April and continued until July 4. What applies to the terminal shoot is even more applicable to the lateral branches where long growth is not as vigorous. Furthermore, the same relation exists between young and old trees. In the latter growth in length must have been completed by July 4, so that it may be concluded that in white pines in the vicinity of Ithaca, growth ceases during the early part of July.[14]

Before proceeding to a review of the results of others, perhaps a brief discussion of the elongation of the leaf is appropriate here. Only one observation was made in regard to this point, but fortunately the date was July 4, so that it offers a chance for comparison between growth of shoots and leaves. The leaves on the

[14] MEISSNER (22) has noted the formation of the so-called "Johannistriebe" in rare cases in white pine. Bud formation occurs in the normal way, but in such cases some buds continue growth the same year. This peculiarity has been noted in many dicotyledonous fruit trees, but is rare in conifers.

terminal shoot of the season were compared with those of the preceding season as to length (not thickness), and the results tabulated in table M in inches. Only 6 trees were examined, so that we cannot expect the uniformity that more extended observations would offer; still, the results are of value in that they lead to a general conclusion.

TABLE M

TABLE OF LONG GROWTH IN NEEDLES

Date	Needle length		Difference	Growth percentage	Average growth percentage
	Old	New			
7-4-1913....	3.75	2.38	137	63	63
7-4-1913....	2.75	1.75	100	63	63
7-4-1913....	2.88	2.00	88	69	63
7-4-1913....	2.88	2.13	75	73	63
7-4-1913....	2.75	1.13	62	41	63
7-4-1913....	2.38	1.75	63	73	63

Elongation in the needles had not ceased on July 4; in no case was it three-fourths completed, as a reference to the table will show. Assuming that elongation in the needle is contemporaneous with elongation in the shoots,[15] that is, that it began on April 26, it follows that during a period of 69 days the needles had attained on an average 63 per cent of the average growth of the preceding season. Assuming again that the rate of elongation was the same during the rest of the season, we may compute roughly the period necessary for the needles to complete their growth, that is, growth in length in the needles would be completed about 40 days after July 4, that is on August 13. It is reasonable to assume from the data on the 6 trees that the elongation of white pine needles ceases somewhere about the middle of August.

If we correlate the results given above with those which have been previously given, we arrive at the following interesting conclusions for white pine in the vicinity of Ithaca. Growth in thickness (secondary thickening) begins in white pine before the elongation of aerial parts, either of shoots or needles. Elongation of shoots and needles begins simultaneously. The elongation of

[15] Field observation substantiated this assertion.

the shoots ceases during early July, while that of the needles continues well into August.

Comparative studies of the growth in length of shoots and needles have been made by others. WIELER (39) found, for example, that in the needles growth was completed in *Pinus montana* at the beginning of July, in *Pinus austriaca* at the end of August, in *Pinus silvestris* by the end of July and the beginning of August, in *Pinus Strobus* during the course of August. Growth of the needles in *Pinus*, according to his observations, varies with the species. MEISSNER (22) likewise noted that growth of needles of a number of species of pine varied, especially that of *Pinus silvestris*. While he gives no exact date for the termination of growth of needles in the species, he states that growth in length of the terminal shoot ceased about the middle of July, and in all cases the growth of the needles continued later than that of the shoot. Whether all species of *Pinus* agree in this respect remains yet to be determined; white pine has proved no exception to the rule.

Primary growth in underground parts

The detection of primary growth in underground parts is in some species attended with obstacles which are well nigh insurmountable. Often the new tissues are little or not at all differentiated from those of the preceding year, and in such cases it is very difficult to detect the beginning of growth in length in the spring. This is the case in white ash, where little coloration results, so that it is quite impossible to separate new and old parts of the root. Fortunately, in the Coniferae this does not apply, for within a space of 1 cm. marked brown coloration appears, so that new growth can be detected without any difficulty. Furthermore, after the cessation of growth in the autumn, this brown mantle approaches nearer the root tip, so near in fact that one can be reasonably sure as to the presence of new growth.

The first observations on root growth were made on April 26, 1913. Roots were obtained from 3 and 4 year white pine specimens in the nursery. The frost had been out of the upper soil layers for only a short time, yet evidences of growth were to be seen in many of the root tips in the shape of small white translucent

protuberences 1 mm. or so in length. Very little elongation had occurred, but clear evidences of its inception were to be seen. Growth in length had already begun.

This early study, as well as the subsequent ones, also brought out another interesting point in regard to white pine roots, namely that there are two kinds of roots underground, just as there are two kinds of branches above. This is well brought out in fig. 11, where long roots and short roots are plainly visible. The short roots occur irregularly on the sides of the long roots, either singly or in tufts of varying size. Where the latter occur, they arise through the forking of a normal short root; this is repeated a number of times and each branch remains short and acquires a growing point of its own. Occasionally one of the apices in the tuft grows out into a long root, but the majority of them remain short, function for a time, but eventually die and disappear as the diameter of the long root increases. Other workers have already noted the same condition in white pine roots. BÜSGEN (4) has described it in some detail, and adds further that mycorhiza are found in the long roots, while they are entirely lacking in the short roots.

Data bearing on root growth were next obtained on May 10. Roots were taken from a thrifty young pine about 12 years old, which from its position on the north bank of Fall Creek near Varna, N.Y., was admirably fitted for the purpose in hand. The creek had partially undermined the sandy bank, and root apices were readily obtained by digging back into the bank. Some of these are represented in fig. 12. The new growth had already attained a length of two inches in many cases, and, as seen in the figure, was sharply marked off from that of the preceding year. This is due to the fact that in the last cork formation (as well as secondary thickening) had occurred, and the thick primary cortex which forms the bulk of the thickness in the new growth was entirely lacking. Browning of the tissues, a peculiarity already described, had also started in the new growth, as the root tip at the extreme right bears evidence. Lateral roots in the form of translucent dots were just appearing on the sides of the growth of the preceding season. It was quite impossible on May 10 to make comparative notes of root growth in 1912 and 1913, inasmuch as the point of origin of growth in the spring of 1912 was not evident. All trace

of yearly elongation is lost after the first year. No attempt was made to trace rapidity of growth of white pine roots. Roots from the same tree on May 30 exhibited an average growth of 4–5 inches, but no other material was secured. We can only infer from the data at hand that growth in length began as early as April 26, possibly much earlier.

The results of others in regard to the duration of root growth are interesting in this connection. RESA (27), after repeated observations on root growth, came to the conclusion that there are in all roots two periods of root development, one in the spring, which occurs mainly before the unfolding of the leaves, and a second during September and October. The last persisted through the winter in dicotyledons, with many interruptions from time to time, but without complete cessation. In conifers, on the other hand, there was a decided rest period during January and February. WIELER (38) combated RESA'S conclusions and maintained that in the autumn, after leaf fall and the resulting lessened demand for water, no new roots were necessary. PETERSON (26) worked with young and old trees of a number of dicotyledons, as well as specimens of *Picea excelsa*, *Pinus montana*, and *Larix decidua*. His results in every way substantiate those of RESA and contradict the conclusions of WIELER. Among other points explained, PETERSON points out that there is a period of root elongation which may occur in the spring anywhere between February and June. In June, and especially in July, elongation gradually ceases. This is followed by a reawakening in growth in length from August until October and even into November. The author does not state in which period growth is more intense. The researches of BÜSGEN in 1901 (4) and ENGLER in 1913 (6) substantiate in every way those of RESA and PETERSON, so it may be concluded that there are two periods of elongation, and furthermore, that in white pine the first begins in late April and continues into early June or even later.

Secondary growth in underground parts

Secondary growth in roots, as in stems, begins the first season, and once started proceeds in the usual way. Mention has already been made of the variability in white pine roots as regards the

number of xylem rays. The secondary xylem forms between the primary xylem rays and under the primary phloem, and it follows that there are as many secondary xylem areas as there are primary xylem rays. In a young root where the secondary thickening has begun we find the primary and secondary xylem areas alternating with each other (figs. 9 and 10). It is usually not until the following year that the segments unite and complete the ring of cambium.

An unsuccessful attempt was made to find out at just what period in the growing season secondary thickening began in the root. Roots were examined on May 11 and again on May 30 with this object in view. No secondary growth was in evidence in either case in the new tissues, even when, as at the last date, elongation had gone on to the extent of 5–6 inches. In every case, however, where the last formed growth of the preceding season was examined, secondary xylem was in evidence between the poles of the primary xylem, and evidence of a resting period was to be seen, so that it must be concluded that secondary growth occurs later in the growing season than May 30, probably during the second period of activity in the autumn. The cambial segments, however, apparently do not unite over the poles the first year, so that secondary growth the first season is confined to as many separate areas as there are poles.

The course of secondary thickening in the root, once started, is much more irregular than in the aerial parts. The annual rings are usually thickest on the lower side of the roots as they enter the root crown, but all regularity is lost a short distance from the bole. The rings may be narrow here and broad there, and apparently their position in the ground has no appreciable effect; geotropism is not a factor in annual ring formation. False and double annual rings are often present. As RUBNER (28) has pointed out, the cambium may be active on one side of a root and dormant on the other for several years without its vitality being impaired, and this is responsible, in part at least, for the irregularities in growth which arise. Furthermore, the tissues of exposed roots present the same characteristics as those in aerial parts, a peculiarity previously noted by KNY (20). In conclusion, it may be said that roots,

while conservative structures in many respects, exhibit much more irregularity in annual ring formation than do stems.

Summary

1. The winter condition of the secondary cortex and cambium of white pine is similar to that of *Pinus rigida*. The marked differences which occur between the mature bark of white pine and pitch pine are occasioned by changes which take place in the outer cortex (periderm).

2. The cambium varies both in number of cell layers (2–10) and thickness in different parts of a tree. It is smallest in both these respects in the twigs and young branches, and increases gradually in dimensions from the apex downward, until that point is reached in the bole where the last annual ring is the thickest. Thereafter, the decrease in the diameter is not proportional to the falling off in the diameter of the last formed ring.

3. Phloem development continues until late in the autumn, much longer than xylem development. Sieve tubes in all stages of formation occur between cambium and fully formed phloem. The seasonal growth of phloem exhibits little or no compression as late as October first. Subsequently contraction occurs, due to the extreme cold temperatures of winter. All the seasonal growth of phloem is crushed with the exception of the last 6 or 8 transitional tracheids. Compression is greater in the crown than below.

4. The processes of primary thickening and secondary thickening overlap, and both may be going on in closely neighboring spots in the tree at the same time.

5. Growth in white pine is divisible into (*a*) growth without cell division and (*b*) growth with cell division. The first begins as early as March and the elements concerned (phloem) increase in radial diameter from 50 to over 100 per cent. The awakening of growth is due apparently to the rise of soil water with an accompanying increase in temperature.

6. Growth by cell division begins during the last half of April. At the start it is very rapid, and more elements are formed at the inside of the cambium than at the outside. The formation of

new xylem elements follows the same order as in pitch pine, that is, it begins first in the bole at some distance below the apical shoot and spreads upward and downward. As a result, growth at the base of a tree may begin several weeks later than in the crown.

7. The awakening and rapidity of growth is dependent on three factors, moisture, available food (reserve), and temperature. The first two are at an optimum in the spring; the amount of growth therefore is directly proportional to prevailing temperatures.

8. The intensity of growth is a variable factor which changes from day to day and even within a single day. Two periodic optimums of growth intensity occur, one during May and early June, the second in July and August. These vary from time to time at a given height in the tree and follow no definite law.

9. The amount of growth at a definite time and place in the tree is equal to the sum of the prevailing growth intensities by the time each was in force. It is very irregular at different heights in the tree, but the cambium tends to even up discrepancies as the season progresses. The irregularities of growth are manifested not only in the actual dimensions of the newly formed tissues, but also in the xylem elements. Wide discrepancies may occur in closely neighboring trees; in general, larger differences may be expected the greater the disparity in age.

10. Growth is first retarded in the upper portions of the tree; it may continue vigorously below for some weeks longer.

11. Xylem formation goes on very sluggishly in all parts of the tree (the terminal leader excepted) until late September and early October, phloem development as long as temperature permits.

12. The total growth of white pine extends over a period of 5.5 months, growth by cell division between 4 and 5 months.

13. Late wood formation begins during the first half of August; it is associated with a decrease in growth intensity and begins first in the higher parts of the tree.

14. Elongation of new shoots and leaves is simultaneous and begins in early May; it manifests itself only after xylem formation has begun. Growth in length in the shoots ceases about July 1; needle growth may continue until August 15 or even later.

15. White pine has long roots and short roots. Only the first elongate to any extent and often are in symbiosis with mycorhiza. Growth in length begins during the last half of April, in some cases even earlier; no reliable data were obtained regarding its cessation. Secondary growth occurs during the first season and proceeds in the usual way.

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Syracuse University
Syracuse, N.Y.

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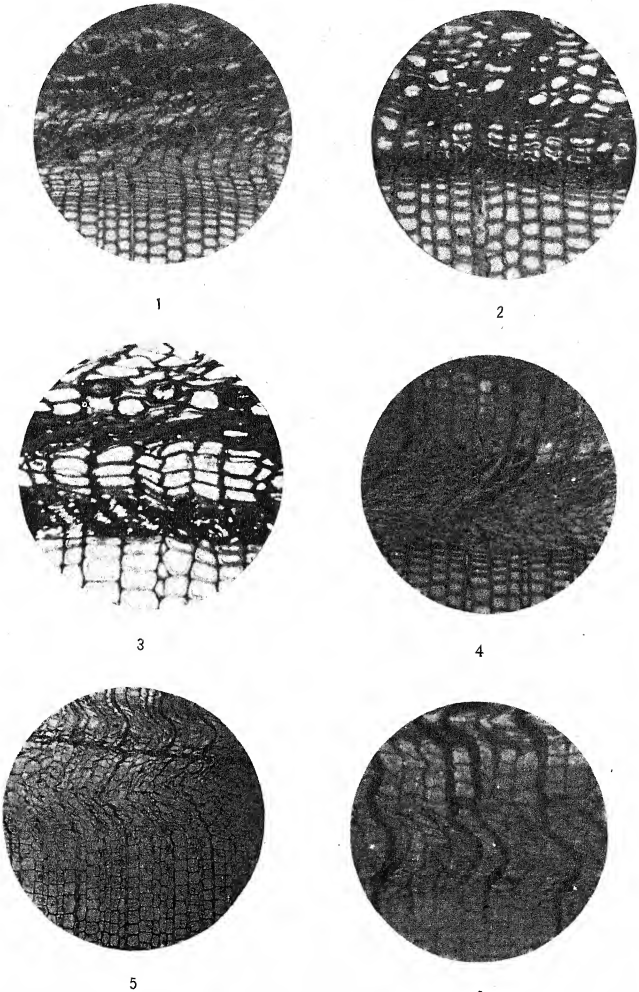

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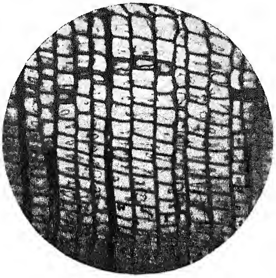
BROWN on PINUS STROBUS



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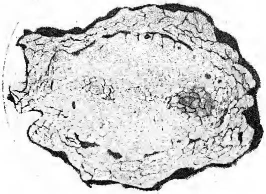

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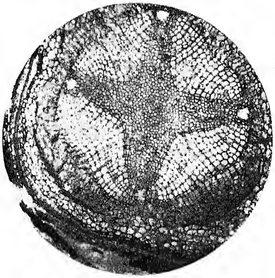

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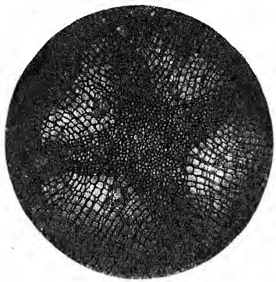

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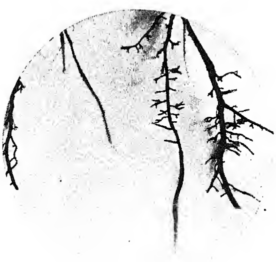

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EXPLANATION OF PLATES XIII AND XIV

Fig. 1.—Cutting III taken from tree I, December 22, 1912; cambium and phloem in the resting condition; ×75.

Fig. 2.—Cutting I taken from tree I, February 20, 1913; phloem (A–B) in winter condition; ×160.

Fig. 3.—Cutting IV, same; contraction from low temperature more restricted; ×160.

Fig. 4.—Cutting I taken from tree I, April 26, 1913; growth taking place very rapidly (*c*, approximate position of the cambium); ×100.

Fig. 5.—Cutting II taken from tree I, May 12, 1913; rapid xylem formation has occurred (*c*, approximate position of the cambium); ×50.

Fig. 6.—Cutting IV taken from tree I, September 19, 1913; transitional forms of tracheids, slow growth; ×110.

Fig. 7.—Cutting III taken from tree I, September 26, 1912; autumnal condition of the phloem; ×200.

Fig. 8.—Diarch white pine root before secondary thickening; two exarch bundles in process of formation; ×90.

Fig. 9.—Tetrarch white pine root after secondary thickening; ×25.

Fig. 10.—Same enlarged, showing 4 original xylem rays; ×35.

Fig. 11.—Long roots and short roots of white pine, May 10, 1913.

Fig. 12.—New growth of long roots, May 14, 1913.

EXTREME ALTERATIONS OF PERMEABILITY WITHOUT INJURY

(WITH FOUR FIGURES)

W. J. V. Osterhout

It has been pointed out in a previous paper[1] that in the opinion of some writers permeability is a relatively fixed property of the cell, and that it is altered only as the result of injury; the alteration is then irreversible. Others assume[2] that there are reversible changes in permeability which may form a normal part of the activities of the cell. In view of the fact that such changes may control metabolism, it seemed important to establish the truth or falsity of this assumption by rigorous proof.

This was successfully accomplished by the use of quantitative methods. The previous paper contained a brief statement of some of the results obtained; the present paper adds important data and describes subsequent experiments in which an extreme range of permeability was attained and very rapid changes were investigated.

The permeability was measured by determining the electrical resistance of living tissues of *Laminaria saccharina* by a method which has been previously described.[3]

It has been shown that the electrical resistance of the living tissue falls rapidly where it is transferred from sea water to a solution of NaCl of the same conductivity, and that within certain limits this effect is reversible. Tissue which in sea water had a resistance of 1020 ohms[4] was placed in a solution of NaCl 0.52M which had the same conductivity as the sea water. In the course of five minutes the resistance fell to 830 ohms. The tissue was replaced in sea water; the resistance soon rose to normal and so continued during the remainder of the day.

[1] Science N.S. 36:350. 1912.

[2] Cf. Höber, Physikalische Chemie der Zelle und Gewebe. Kap. 7 und 10. 1911.

[3] Science N.S. 35:112. 1912.

[4] All readings were taken at 18° C. unless otherwise stated.

As the electrical conductivity of the tissue is a measure of the permeability of the protoplasm to ions, we may calculate the percentage of increase of permeability by finding the change in conductivity. In the present instance it is more convenient to use the change in conductance without reducing this to specific conductivity. The resistance at the start was 1020 ohms, but this includes the resistance of the apparatus with its contained sea water. Evidently this should be subtracted from the total resistance; the remainder will be called the *net resistance*.[5]

In this case the resistance of the apparatus was 250 ohms. The net resistance of the tissue at the start, therefore, was $1020-250=770$ ohms; the net conductance was $1\div 770=0.00130$ mho. At the end of five minutes in NaCl the net resistance was $830-250=580$ ohms; the net conductance was $1\div 580=0.00172$ mho. The increase in permeability, therefore, amounts to $0.00172-0.00130=0.00042$ mho, or 32.3 per cent.[6]

It might be objected that this increase in conductance was not due to an increase in permeability but to an increase in the ions of sodium chloride, to which the tissue might be assumed to be normally more permeable than to some of the other ions of the sea water. This, however, cannot be the case, as is shown by the following experiment. Tissue having a resistance of 1020 ohms was placed in a mixture of 1000 cc. NaCl 0.52M+20 cc. $CaCl_2$ 0.278M. The resistance remained at 1020 ohms in this mixture. The tissue was then transferred to NaCl 0.52M for five minutes. At the end of this time the resistance had fallen to 860 ohms; on being placed in sea water the resistance rose to the normal and so remained for some time.

In this case the resistance of the apparatus was 230 ohms. The net resistance at the start was $1020-230=790$ ohms; and the net

[5] In the previous paper it was suggested that the tissue should be killed, that the resistance of the apparatus should be measured while the dead tissue remained in it, and that this should be subtracted from the total; the remainder was called the net resistance. It seems better, however, to take the resistance of the apparatus after the tissue has been removed, to subtract this from the total, and call the remainder the net resistance.

[6] Complete recovery after such a large increase of permeability is not always obtainable unless the material is in good condition and is freshly collected. Even in such material a lot will occasionally be found which shows poor recovery.

conductance $1 \div 790 = 0.00127$ mho. After treatment with NaCl the net resistance was $860 - 230 = 630$ ohms, and the net conductance was $1 \div 630 = 0.00159$ mho. The increase in net conductance, therefore, was $0.00159 - 0.00127 = 0.00032$ mho, or 25.2 per cent. The increase in the percentage of sodium ions was only 2 per cent, while the content of chlorine ions remained unchanged. It is evident, therefore, that there was a great increase in permeability.

In order to see whether this increase of permeability is accompanied by injury, an experiment was made in which the same piece of tissue was exposed to the action of NaCl several times during the same day. The resistance of the tissue in sea water was 1010 ohms; after five minutes in NaCl the resistance fell to 880 ohms; the tissue was then placed in sea water and a reading ten minutes later showed that the resistance had risen to 1010 ohms. During the next 95 minutes it showed no change. It was then placed in NaCl for five minutes and the resistance fell to 870 ohms. It was replaced in sea water; a reading taken ten minutes later showed that it had returned to normal, where it remained for 90 minutes without change. It was then placed in NaCl for five minutes. The resistance fell to 900 ohms and returned to normal during the ensuing ten minutes in sea water. After 105 minutes in sea water, during which no change occurred, it was again exposed to NaCl for five minutes. The resistance fell to 870 ohms and returned again to normal during the following ten minutes in sea water. On the following day its resistance was only 30 ohms below the resistance of the control, which at the beginning of the experiment was 1040 ohms. The results are presented graphically in fig. 1.

The successful outcome of this experiment lead to an attempt to carry on such an experiment for several days in succession, giving the tissue one treatment daily with NaCl. The material was selected with especial care. The fronds were fairly thick, without reproductive organs. The experiment was made at Woods Hole, Mass., in July, at which time such fronds may be easily obtained. The disks cut from these fronds were slightly curved, so that when placed in the apparatus they separated spontaneously, thus allowing the running sea water in which they were kept to

circulate freely between them. Care was taken to keep them only about two-thirds submerged, so that they had free access to air without any risk of drying.

The tissue had in sea water a resistance of 1020 ohms at 20° C. As the temperature of the sea water varied but slightly from this during the experiment, all readings were taken at 20° C. On being placed in NaCl 0.52M at this temperature, the resistance fell in five minutes to 890 ohms; it was then placed in sea water and a reading taken ten minutes later showed that it had risen again to the normal. The resistance of the apparatus was 240 ohms; hence

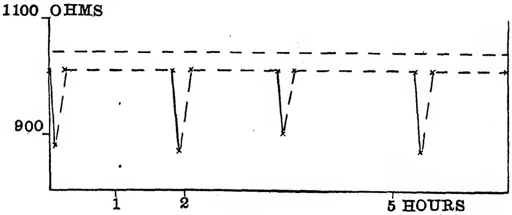

FIG. 1.—Alterations of permeability shown by curves of the electrical resistance of *Laminaria saccharina* in NaCl 0.52M (unbroken line) and in sea water (dotted portion of the curve); the horizontal dotted line (above) shows the resistance of the control.

the net resistance at the start was 1020−240=780 ohms, and the net conductance 1÷780=0.00128 mho. The net resistance after treatment with NaCl was 890−240=650 ohms, and the net conductance 1÷650=0.00154 mho. The increase in permeability, therefore, was 0.00154−0.00128=0.00026 mho, or 20.3 per cent.

The tissue was then placed in running sea water for 22 hours, with the precautions mentioned above. At the end of 22 hours the resistance was 1020 ohms at 20° C. An exposure of five minutes to NaCl resulted in a drop to 920 ohms, with complete recovery within ten minutes. The same treatment was given once each day for 15 days. On the tenth day the resistance began to fall off, but as this falling off was also shown by the control, which remained in

sea water through the experiment, it was not due to the sodium chloride but to other causes. The results are shown in table I and fig. 2.

TABLE I*

Day	Resistance before exposure	Fall of resistance after 5 minutes in NaCl	Recovery	Control in sea water
1	1020	130	Complete	1030
2	1020	100	"	1030
3	1020	110	"	1030
4	1020	140	"	1030
5	1020	120	"	1030
6	1020	120	"	1030
7	1020	100	"	1030
8	1020	130	"	1030
9	1020	120	"	1030
10	1000	120	"	1020
11	1000	110	"	1010
12	980	100	"	1010
13	960	110	"	970
14	950	120	"	960
15	930	100	"	950

* All readings were taken at 20° C.

Electrolytes may also cause a reversible decrease in permeability. The simplest way of demonstrating this is by means of the following very striking experiment. The resistance of a cylinder of living tissue in sea water was found to be 750 ohms. It was tested an hour later and found to be the same. Sufficient lanthanum nitrate (8.7 gm. to 1000 cc. sea water) was then added in solid form to make its concentration[7] in the sea water 0.01M. After five minutes the resistance rose to 900 ohms. As the resistance of the apparatus was 250 ohms, the net resistance before the addition of lanthanum was $750-250=500$ ohms, and the net conductance $1\div 500=0.002$ mho. After treatment with lanthanum nitrate, the net resistance was $900-250=650$ ohms, and net conductance $1\div 650=0.00154$ mho, a loss of 23 per cent.

[7] The concentration was reduced by the precipitation of a small amount of lanthanum sulphate; this had practically no influence on the subsequent result, since the outcome is the same if we use in place of sea water a mixture of 1000 cc. NaCl 0.52M+ 20 cc. $CaCl_2$ 0.278M, in which case no precipitate is formed. It should be noted that the addition of lanthanum chloride has the same effect as the addition of lanthanum nitrate.

In order to ascertain whether this change in permeability is reversible, the tissue was replaced in sea water. In the course of an hour its resistance returned again to the original condition.[8] The experiment was then repeated three times on the same lot of material with practically the same result; it was then allowed to stand over night in sea water. On the following day there was no

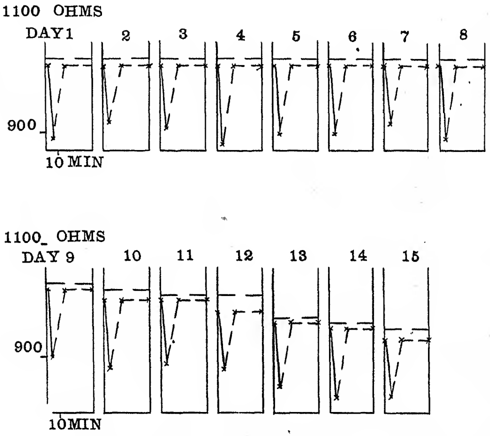

FIG. 2.—Alterations of permeability shown by curves of the electrical resistance of *Laminaria saccharina* in NaCl 0.52M (unbroken line) and in sea water (dotted portion of curve); upper dotted line, control in sea water.

appearance of injury, and its resistance was the same as that of the control, which had remained in sea water throughout the experiment. The tissue was then placed in the sea water plus lanthanum and left until its resistance had increased 100 ohms; it was then put back into sea water and left until the resistance fell to nearly normal. This was repeated three times, and the tissue

[8] If the material is left in sea water plus lanthanum nitrate the increased resistance is maintained for a long time unaltered.

was then allowed to stand over night in sea water. On the third, fourth, and fifth days the same experiment was repeated four times. On the fifth day the tissue appeared to be in as good condition as the control, and had a resistance which was slightly higher. There

TABLE II*

	Resistance at start of exposure	Resistance rose in sea water $+La_2(NO_3)_6$ to	Recovered to
Day 1—			
Exposed during 5 minutes	750	900	750
	750	870	750
Recovered during 55 minutes	750	900	750
	750	850	750
Control = 730			
Day 2—			
Exposed during 20 minutes	700	860	710
	710	830	710
Recovered during 100 minutes	710	850	710
	710	840	710
Control = 690			
Day 3—			
Exposed during 30 minutes	690	790	710
	710	800	720
Recovered during 100 minutes	720	790	710
	710	790	700
Control = 660			
Day 4—			
Exposed during 30 minutes	670	760	680
	680	750	670
Recovered during 100 minutes	670	780	680
	680	770	680
Control = 650			
Day 5—			
Exposed during 40 minutes	660	760	660
	660	780	660
Recovered during 120 minutes	660	770	660
	660	760	660
Control = 650			

* All readings were taken at 20° C.

was no reason, therefore, to suspect that the changes in permeability had been attended by any injurious effect. The results are shown in detail in table II and fig. 3.

Similar experiments were performed in which calcium chloride was used in place of lanthanum nitrate. In this case 3.3 gm.

$CaCl_2$ were added to each 1000 cc. of sea water. Owing to the fact that the rise in resistance took place more slowly[9] than when lanthanum was used, the experiment was performed twice on each of the five successive days. On the sixth day the material was in as good condition as the control and had the same resistance.

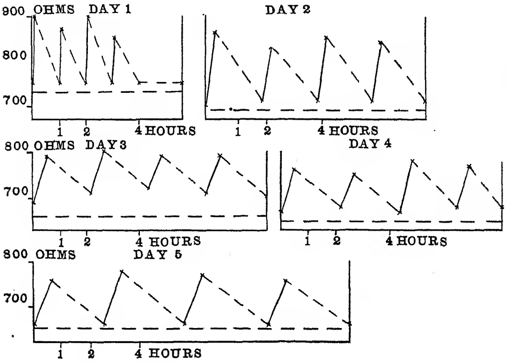

FIG. 3.—Alterations of permeability shown by curves of the electrical resistance of *Laminaria saccharina* in sea water, and in 1000 cc. sea water+2.6 gms. $La_3(NO_3)_6$ (=0.01M); the same lot of material was exposed four times daily on five successive days to the action of 1000 cc. sea water+$La_2(NO_3)_6$; unbroken part of curve, resistance in sea water+$La_2(NO_3)_6$; dotted part of curve, resistance in sea water; lower horizontal dotted line, control in sea water.

It is evident, therefore, that the permeability may be greatly decreased and then restored to the normal several times on five successive days without any trace of injury. Further experiments showed that the permeability may be alternately increased and decreased twice daily for five days without injury. The amount of increase and of decrease was about the same as in the experiments just described.

[9] If in place of solid $CaCl_2$ a strong solution is added, the rise is more rapid and reaches a higher figure.

Experiments on dead tissue (killed by heat or by formalin or allowed to die a natural death) showed that the results described above are due entirely to the living cells.

A very marked decrease of permeability may be produced by a considerable variety of other salts. The addition of these salts in solid form simultaneously increases the conductivity of the solution and decreases the conductivity of the tissue. This affords the most convincing proof that the change in the conductivity of the tissue in these experiments cannot be due to any cause other than a change in permeability; for the concentration of the ions of the sea water remains unchanged, and if they were able to penetrate as freely as they did before the addition of the salt, the resistance would not increase. It would, in fact, diminish on account of the increased conductivity of the solution held in the cell walls, as is clearly shown by experiments on dead tissue.

It may be remarked incidentally that these experiments effectually dispose of the possible objection that the current passes between the cells but not through them. Were this objection well founded, the decrease in conductivity could be explained only as the result of a decrease in the size of the spaces between the cells. This decrease could not be brought about except by greatly reducing the thickness of the cell walls. Both macroscopic and microscopic measurements show most conclusively that this does not occur. The contrary effect would be produced by the addition of salts in solid form, for they would tend to produce plasmolysis and thereby to increase the space between the cells.

As these remarkable changes in permeability seemed to produce no bad effects, it occurred to the writer to see whether the protoplasm could endure still more violent alterations without permanent injury. In order to test this the following experiment was performed. A lot of tissue was found to have in sea water a resistance of 1010 ohms. It was placed in $CaCl_2$ 0.278M, which had the same conductivity as the sea water. At the end of ten minutes a reading was taken which showed that the resistance had risen to 1500 ohms. The material was then placed in NaCl 0.52M, which had the same conductivity as the sea water; at the end of ten minutes the resistance was 880 ohms. The experiment was con-

tinued by placing the material for ten minutes alternately in $CaCl_2$ and NaCl, with the results shown in table III and fig. 4. After 80 minutes the material was placed in sea water, where it soon regained its normal resistance of 1010 ohms. Twenty hours later the resistance was found to be unaltered and the experiment was repeated. After 80 minutes of alternate exposure to $CaCl_2$ and NaCl, the material was placed in sea water, where it soon regained its normal resistance, which it maintained for three days, when the experiment was discontinued.

TABLE III*

ALTERATIONS IN ELECTRICAL RESISTANCE OF *Laminaria saccharina* EXPOSED FOR 10 MINUTES ALTERNATELY TO $CaCl_2$ 0.278 AND NaCl 0.52M

Day 1			Day 2		
Time in minutes	Solution	Resistance	Time in minutes	Solution	Resistance
0		1010			1010
10	$CaCl_2$	1500	10	$CaCl_2$	1490
20	NaCl	880	20	NaCl	880
30	$CaCl_2$	1470	30	$CaCl_2$	1500
40	NaCl	900	40	NaCl	900
50	$CaCl_2$	1500	50	$CaCl_2$	1460
60	NaCl	860	60	NaCl	900
70	$CaCl_2$	1470	70	$CaCl_2$	1480
80	NaCl	890	80	NaCl	880
95	Sea water	1010	95	Sea water	1010
115	" "	1010			
Control in sea water 990			Control in sea water 990		

* All readings were taken at 18° C.

The resistance of the apparatus was 240 ohms. The net resistance of the tissue at the start, therefore, was $1010-240=770$ ohms, and the net conductance $1\div770=0.00130$ mho. After the first exposure to $CaCl_2$, the net resistance was $1500-240=1260$ ohms, and the net conductance was $1\div1260=0.00079$ mho. The loss in conductance was $0.0013-0.00079=0.00051$ mho, or 39.2 per cent.

After the first exposure to NaCl the net resistance was $880-240=640$ ohms, and the net conductance $1\div640=0.00156$ mho.

This was greater than the normal by 0.000156−0.0013=0.00026 mho, or a gain of 20 per cent.

The fact that protoplasm is able to endure such violent alterations of permeability throws a new light on the normal life processes of the cell. In the course of metabolism a great variety of substances are produced which affect the permeability of the protoplasm. Since it is clear that the permeability may be increased or

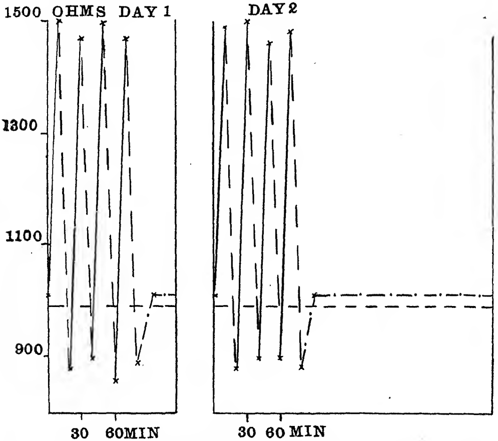

FIG. 4.—Extreme alterations of permeability shown by curves of electrical resistance of *Laminaria saccharina* in $CaCl_2$ 0.278 M (unbroken line), in NaCl 0.52 M (dotted portion of curve), and in sea water (dotted line with points); horizontal dotted line, control in sea water.

decreased 30 per cent or more without rendering a return to normal permeability impossible, it is evident that considerable fluctuations in permeability may form a normal part of the life processes of the protoplasm. In this way the whole course of metabolism may be controlled, since this evidently depends on the exchange of substances between the cell and its environment.

Summary

Results obtained by the use of quantitative methods prove that the permeability of protoplasm may be greatly increased or diminished without injury. A rapid alternation of increase (amounting to 20 per cent above normal) and decrease (amounting to 39 per cent below normal) did not produce injury.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

BRIEFER ARTICLES

APOGAMY IN NEPHRODIUM HERTIPES

PRELIMINARY NOTE

Several cultures of *Nephrodium hertipes* were made beginning December 14, 1913. The spores were collected from plants grown in the university greenhouse and were sown on sphagnum placed in small stender dishes and then saturated with a 0.1 per cent Knop's solution. Before the spores were sown, the dishes containing the medium were thoroughly sterilized in an oven. The prothallia were grown under favorable conditions of nutrition, illumination, moisture, and temperature. While the sex organs were developing on the prothallia of other species under these conditions, it was observed that only an occasional prothallium of this fern produced antheridia. Archegonia were never seen on any of the prothallia.

In April 1914, many of the prothallia in the oldest culture were typically heart-shaped. A microscopical examination made at this time showed that embryos of apogamous origin had begun their development. When the embryo is about to make its appearance, usually a small light area, just back of the apical notch, is formed. This appearance is due to the fact that only a few chromatophores are present in the cells of this part of the prothallium as compared with the larger number present in the neighboring prothallial cells. Tracheids appear in the paler portion of the prothallium just described. The light region gradually increases in extent. Where the tracheids are formed, a compact mass of cells is produced which develops into the apogamous embryo. A foot is never formed by the developing embryo. The primary leaf first makes its appearance, then the primary root, and later the stem. In this order of development of the parts of the embryo, *Nephrodium hertipes* resembles some other apogamous ferns thus far described. In some cases, however, the root appears before the leaf. On large, much-branched prothallia several apogamous embryos may begin to develop. Some of these abort and seldom more than one embryo on a prothallium reaches an advanced stage.

Occasionally cylindrical growths, often containing tracheids, are produced from the prothallia. As growth proceeds, these sporophyte-like portions flatten out at the growing ends and assume the form of

ordinary prothallia, which may in their turn produce apogamous embryos. In all my cultures apogamous embryos have been produced in large numbers. From observations so far made it appears that all the prothallia may produce such embryos. Some of the young sporophytes have been grown to a height of several inches and are apparently normal in every respect.

An investigation of the nuclear history of this species led to the discovery of cell and nuclear fusions in the sporangia, similar to those described for the first time by Miss RUTH ALLEN in *Aspidium falcatum*, and not thus far described in any other fern. Nothing unusual has been observed in the early stages in the development of the sporangia. By four successive divisions 16 cells are formed from the primary sporogenous cell. These cells instead of functioning as spore mother cells, as do the cells of the corresponding cell generation in the sporangia of most of the Polypodiaceae, fuse in pairs. Sometimes the wall between two neighboring cells completely disappears before the fusion of the nuclei. Frequently only a portion of the walls disappears before the fusion of the nuclei is completed, but as a rule they wholly disappear later.

The 8 cells produced by the fusions just described are the ones that function as the spore mother cells. Heterotypic and homoeotypic divisions occur, forming typically 32 spores. The mature sporangium, however, frequently contains fewer than 32 spores. Irregularities occurring in sporogenesis may account for the presence of the smaller number of spores in a sporangium. Spore mother cells in synapsis, various stages in the divisions of the spore mother cells, and tetrads are sometimes found at the same time in a single sporangium. This is out of harmony with the usual course of events in fern sporangia, and it is possible that some of the cells in the earlier stages of development may fail to complete their division. Occasionally cell and nuclear fusions are not completed. It is highly probable that the number of spores in a sporangium is frequently reduced by these irregularities and abnormalities among the spore mother cells.—W. N. STEIL, *University of Wisconsin, Madison, Wisconsin.*

CURRENT LITERATURE

BOOK REVIEWS

Experimental genetics

When one of the foremost investigators in any science has the additional ability which enables him to write a clear, well balanced textbook, it is only just that public appreciation should increase in geometrical proportion, for such proficiency is rare. For this reason the writer feels sure that he voices a unanimous sentiment among geneticists in thanking Dr. BAUR for bringing up to date his *Introduction to experimental genetics*. The original edition, published in 1911, probably had fewer defects in judgment of values than any of the textbooks on the subjects that have been issued so frequently since 1900. The new edition,[1] with 100 added pages, fully sustains this opinion. And such a seemingly odious comparison with other books is no disparagement of their value, for most other volumes on genetics have treated only particular phases of the subject. If any broad criticism can be made of either edition, it is that biometrical and cytological results have hardly been given the space they merit, though the present edition has partially abrogated this deficiency.

The author follows the general plan of the first edition, the additional pages being made necessary because of the numerous investigations of the past two years. The first two chapters lay a foundation for discussing the inheritance of acquired characters. In reality they are concerned with plant physiology and morphology from the genetic standpoint. By making use of elementary biometrical formulas, the changes during ontogeny due to varying external conditions are carefully explained, emphasis being laid on the variation in ability to react to stimuli at different parts of the life cycle. Then follow two chapters in which the more modern experimental attacks on the inheritance of modifications are clearly and logically described and criticized.

The next 100 pages are filled with Mendelian results. The elementary principles are described well and many new illustrations are used, but the more recent work is not adequately treated. For example, the marvelous work of MORGAN in analyzing the germ plasm of *Drosophila* is hardly mentioned. One is the more astonished at this omission when he sees that several pages are given over to BATESON's theory of partial coupling, a theory that cannot compare with MORGAN's for ingenuity, reasonability, and logical agreement with facts.

[1] BAUR, E., Einführung in die experimentelle Vererbungslehre. Zweite Auflage. viii+401, mit 131 Textfiguren und 10 farbigen Tafeln. Berlin: Gebrüder Borntraeger. 1914.

The next chapter discusses the apparent cases of non-Mendelian heredity, inheritance only through the mother, and vegetative segregation in the first hybrid generation. This is followed by some 30 pages on the inheritance of sex, which is not wholly satisfactory on account of the omission of so much recent work from both the cytological and the pedigree culture side. Odds and ends are picked up in the next two chapters. The first is largely an account of the many peculiar results occurring in species crosses to which as yet there is no satisfactory explanation. The other describes graft hybrids and xenia.

The six remaining chapters are rather general in character, and partly for this reason are highly recommended to biologists who are not specialists in genetics. They deal with questions of variation and heredity in a broad way, from the viewpoint of a man thoroughly conversant with all modern investigations, philosophical as well as experimental.

It has been generally understood that an English translation was to appear simultaneously with the German edition. Let us hope that the war will only delay and not prevent its publication.—E. M. EAST.

MINOR NOTICES

A manual of weeds.—The present volume[2] is probably the most extensive and exhaustive weed manual yet published. In fact, the author has taken the term "weed" in its broadest sense and has included many plants not usually regarded as pernicious; for example, the list embraces several of the golden rods, clovers, asters, and roses, and even such trees as the wild black and choke cherries. About 500 species are described in semi-technical terms and three-fourths of them are illustrated by habit drawings. They are arranged under their respective families, but no keys or other means of identification are supplied. This seems to be the greatest defect of the manual and one that might have been rather easily remedied. Both common and scientific names are given, the former including some of the more common synonyms, and the range is made to include all of the United States and Canada. The illustrations, although rather small, will certainly prove to be one of the most useful features of the book, enabling any one with a minimum of scientific training to recognize with considerable accuracy all weedy plants of common occurrence.

Like the other volumes of this series of "Rural manuals" edited by L. H. BAILEY, this manual of weeds will be found useful as a textbook in agricultural colleges, but it makes its strongest appeal to the practical tiller of the soil. In this connection it is gratifying to note that the problems of weed control receive considerable attention, although the importance of rotation of crops seems to be less emphasized than its efficiency deserves.—GEO. D. FULLER.

[2] GEORGIA, ADA E., A manual of weeds. 12mo. pp. xi+561. *figs. 385.* New York: Macmillan. 1914. $2.00.

Fodder and pasture plants.—At times it is difficult for the student of agriculture to obtain at once adequate botanical descriptions and cultural data of plants commonly used for fodder and pasture purposes, while the farmer is frequently poorly informed upon either phase of knowledge relating to the plants he is constantly growing. A recent volume by CLARK and MALTE[3] seems to be particularly well fitted to meet the needs of both student and farmer. Its botanical descriptions of the grasses and clovers usually cultivated are accurate but non-technical, while in addition it furnishes abundant data upon the geographical distribution, cultural conditions, habits of growth, and agricultural value of the plants discussed. Perhaps the best feature of the volume is the admirable series of colored plates depicting the species described with such accuracy that any one, even without botanical training, can have no difficulty in at once recognizing them. In this respect the volume is uniform with the *Farm weeds of Canada* previously noticed in this journal,[4] and it will form a valuable addition to the equipment of the teacher of agriculture as well as a convenient book of reference for the farmer.—GEO. D. FULLER.

Ferns of Washington.—Under this title FRYE and JACKSON[5] have published a small book which is a boon to those who wish to become familiar with the ferns of Washington. It includes the true ferns, water ferns, adders-tongues, grape ferns, horse tails, scouring rushes, club mosses, moss ferns, and quillworts. The writers find 66 species of pteridophytes in the state, of which 30 are Polypodiaceae. These species belong to 24 genera, of which 16 are Polypodiaceae. The work has a key to families, and keys to the genera and species. The families, genera, and species are all described. The habitat and the range of each species is given. In a state comprising such a diversity of regions as does Washington, the distribution within the state would add to the usefulness of the work. It is illustrated with 20 plates made from drawings and photographs, illustrating the principal species treated in the work. This publication will undoubtedly add greatly to the interest in the ferns and their allies in the Northwest.—GEORGE B. RIGG.

NOTES FOR STUDENTS

Biology of Fegatella.—Miss MAYBROOK[6] examined vegetative thalli of *Fegatella conica* found growing in a cavelike hole. In regions of greatest light intensity the thallus showed the structures common to *Fegatella*, but as the

[3] CLARK, GEO. H., and MALTE, M. O., Fodder and pasture plants. 8vo. pp. 143. *pls. 27*. Ottawa: Dept. of Agric., Dominion of Canada. 1913. 50 cents. For sale by Superintendent of Stationery, Government Printing Bureau, Ottawa.

[4] BOT. GAZ. 50:389. 1910.

[5] FRYE, T. C., and JACKSON, MABEL M., The Ferns of Washington. pp. 60. *pls. 20*. Seattle, Wash.: Lowman & Hanford. 1914. Reprinted from Amer. Fern Jour. 3:65–83, 97–108. 1913; 4:6–14, 41–57. 1914.

[6] MAYBROOK, ANNIE C., Note on the biology of *Fegatella conica*. New Phytol. 13:243–249. *fig. 1*. 1914.

light intensity decreased the thallus decreased in size, the air chambers decreased in number per unit area, and chloroplasts appeared in the dorsal epidermal cells. In the region of least light intensity and in dripping water a form was found which showed neither air chambers, ventral scales, nor tuberculate rhizoids. Miss MAYBROOK concludes that the factors responsible for this condition of the thallus are diminished light intensity and excessive moisture. Since none of these plants were in fruit the question of identity naturally is of prime importance. The long series of recently conducted experiments on undoubted *Fegatella conica* by BRYAN in this laboratory show that under extreme conditions of moisture the air chambers can be somewhat modified. BRYAN eliminated neither air chambers nor ventral scales. The reviewer considers the presence of air chambers and ventral scales of such importance in undoubted Marchantiales that he hopes Miss MAYBROOK will place some of the plants under suitable conditions for fruiting in order that there may be no doubt of their identity.—W. J. G. LAND.

Notes from Florida.—HARSHBERGER[7] has written a popular sketch of his journey across the Everglades, promising later to give a detailed account of the plant formations studied. Attention is called to the great lack of scientific knowledge of this region. South Florida is regarded as that portion of the state south of 27°. Brief treatment is given the plant and animal life, agricultural possibilities, and other topics.

BESSEY[8] has given a brief description of the hammocks, as they are seen about Miami, contrasting them with the pine lands and with the Everglades. Reference is made to a number of the more interesting species, and the cause of the sharp contrast between the vegetation of the pine lands and that of the hammocks is discussed.

In a steamboat ride up the Apalachicola River, R. M. HARPER[9] noted a considerable change in the bank vegetation in the progress of the journey. Among the possible explanations suggested for this common phenomenon, the chief place is given to the probability that the upstream plants require or tolerate greater fluctuation in level than do the plants of the estuarine swamps, in which, of course, the seasonal changes in level are small.—H. C. COWLES.

An ecological study of weeds.—Weeds have been largely neglected by ecologists and phytogeographers, who for the most part have concerned themselves with the more primeval types of vegetation. For several years Miss BRENCHLEY has been making observations on the soil relations of weeds, and

[7] HARSHBERGER, J. W., South Florida; a geographic reconnaissance. Bull. Geog. Soc. Phila. **10**:37–47. *figs. 10*. 1912.

[8] BESSEY, E. A., The hammocks and everglades of southern Florida. Plant World **14**:268–276. *figs. 2*. 1911.

[9] HARPER, R. M., The river-bank vegetation of the lower Apalachicola, and a new principle illustrated thereby. Torreya **11**:225–234. *fig. 1*. 1911.

she has made three reports on her studies.[10] The work has been carried on in Southern England, and careful effort was made to compare conditions in several different counties. It is concluded that some weeds are ubiquists, occurring on all soils, whereas other weeds are definitely symptomatic. Symptomatic species are most in evidence on chalk, although it is to be noted that most of the weeds which are calcifuges in Bedfordshire are calcicoles in Wiltshire and Somerset. Examples of such reversal are *Chenopodium album* and *Bartsia Odontites; Poa annua* is about the only consistent calcifuge observed. In one place a mingling of chalk plants and "acid plants" was explained by a non-calcareous surface soil overlying a chalk subsoil. In some cases the character of the crop influences the weed population, as in certain leguminous seed crops. Some plants, as the mayweeds (*Anthemis, Matricaria*), are impatient of competition.—H. C. Cowles.

Morphology of Macroglossum.—*Macroglossum* is a new generic type of the Marattiaceae described in 1909 by Copeland from material obtained from Borneo. A recent visit to this region enabled Campbell to secure material of this fern, and he has now published an account of its structure and affinities.[11] The genus now comprises two species, the second one having been found growing in the botanical gardens at Buitenzorg, but of unknown origin. The species studied is a large plant, the leaves reaching sometimes a length of 4 meters. It belongs to the *Angiopteris* group, related apparently most nearly to *Archangiopteris*. It differs much in general appearance from *Angiopteris*, as well as in its much elongated and partially immersed sori. The sporangia also are smaller and very much more numerous than those of *Angiopteris*. The gametophyte may reach a length of 3 cm., and branching is not uncommon. The antheridia occur on both surfaces, and the number of sperm mother cells is probably greater than in any other of the Marattiaceae. The embryo develops a conspicuous suspensor, as in *Danaea*. The author also describes certain anatomical details, comparing them with those of the other Marattiaceae.—J. M. C.

Leaf-sheath trichomes in grasses.—In many grasses, especially those of xerophytic and alpine habitats, the leaf sheaths do not decay immediately after death. Instead of this they remain, forming a sort of mantle about the young sheaths. That this feature is especially characteristic of xerophytic grasses was noted in 1890 by Hackel, who regarded the mantles as having a protective function, tending to reduce transpiration. H. Brockmann-Jerosch[12]

[10] Brenchley, Winifred E., The weeds of arable land in relation to the soils on which they grow. Ann. Botany **25**:155–165. 1911; **26**:95–109. 1912; **27**:141–166. 1913.

[11] Campbell, D. H., The structure and affinities of *Macroglossum Alidae* Copeland. Ann. Botany **28**:651–669. *pls. 46–48. figs. 8.* 1914.

[12] Brockmann-Jerosch, H., Die Trichome der Blattscheiden bei Gräsern. Ber. Deutsch. Bot. Gesells. **31**:590–594. *pl. 1.* 1914.

calls this interpretation in question. For example, in *Festuca spadicea* these persistent sheaths are found in the soil, where protection from transpiration is of little importance. A more striking observation was made on *Festuca varia*, a species that grows in winter while the soil about its roots is still frozen. Thinking that there might be absorptive organs beneath the mantles, the author finds that downward-pointing hairs are present in this position in many of these grasses. Mostly from such circumstantial evidence, BROCKMANN-JEROSCH postulates that these hairs are water-absorptive organs. Such an observation needs experimental corroboration, as the author frankly recognizes.—H. C. COWLES.

Soil studies.—E. E. FREE[13] of the U.S. Bureau of Soils has brought together the essential features of our knowledge of soil physics in admirable form for use by physiologists and ecologists. The material is treated under the following heads: the physical condition of soils, the movements of soil water, soil water and the plant, the physical constants of soils, and soil temperature.

FREE has also published an elaborate paper on soil movement by wind.[14] While this treatise will be of value in the first instance to physiographers, it will also be of great interest to all ecologists who are interested in the vegetation of such wind deposits as sand dunes or loess. Among the topics treated are the mechanics of wind translocation, drifting sand and sand dunes, dust storms and dust falls, atmospheric dust, geologic formations of eolian origin, and volcanic dust as soil material. At the close is a remarkably complete bibliographical index of eolian geology; in the compilation of this index FREE was aided by S. C. STUNTZ.—H. C. COWLES.

Defoliation and wood structure.—In recent years many trees of the European larch in the English Lake District have been repeatedly defoliated by the large larch sawfly. Some of the trees have been studied by HARPER[15] to determine the influence on wood structure. Such defoliation means starvation to a greater or less degree, and starvation affects both the amount of growth and the structure of the wood. In the lower parts of the tree, where the rings ordinarily are narrower than they are above, growth may cease altogether; higher up, where there is more growth, the rings may not completely encircle the tree. Even before this effect is seen, there is a reduction in the wall thickening of the autumn wood. This situation is related to an actual lack in the foods necessary to build up these tissues to the usual amount.—H. C. COWLES.

[13] FREE, E. E., Studies in soil physics. Plant World 14:29–39, 59–66, 110–119, 164–176, 186–190. 1911.

[14] FREE, E. E., The movement of soil material by the wind. U.S. Bureau of Soils, Bull. 68. pp. 272. *pls. 5.* 1911.

[15] HARPER, A. G., Defoliation: its effects upon the growth and structure of the wood of *Larix*. Ann. Botany 27:621–642. *pls. 2. figs. 2.* 1913.

Vegetative reproduction in Selaginella.—Miss BANCROFT[16] has investigated the reproductive "tubers" of two species of *Selaginella* from India. In *S. chrysocaulos* there occur budlike structures at the tips of some of the vegetative branches; while in *S. chrysorrhizos* the stem apices forming the "buds" repeatedly fork, rhizophores often occurring in the fork between two branches. Miss BANCROFT investigated the behavior of both these reproductive structures, which differs in details, since in one of the species the "tubers" remain at the surface of the ground; while in the other they are developed underground, at the ends of filamentous vegetative branches.—J. M. C.

Anatomy of some xerophilous ferns.—MARSH[17] has made an anatomical study of certain xerophilous species of *Cheilanthes* and *Pellaea*, material having been obtained chiefly from the United States. Such well marked leaf "adaptations" as hairs or scales on the lower surface, inrolled margins, thick cuticle, and palisade parenchyma are described. The xylem features are discussed in detail, and one of the interesting conclusions is that "the petiolar structure, the stem anatomy, and the greater output of spores per sporangium, all point to *Cheilanthes Fendleri* as a near approximation to an ancestral type, from which *C. gracillima* and *C. lanuginosa* have been derived."—J. M. C.

Sphagnum bogs of Alaska.—RIGG[18] has noted the peculiarities of the flora of some Alaskan peat bogs and finds that while sphagnum occurs in many different habitats in Alaska, only where there is an absence of drainage do bogs accompany it. The peat in the bogs visited had a maximum depth of only 2.5 ft. Aside from the sphagnum, *Empetrum nigrum* is the most abundant and uniform in its occurrence, but *Ledum palustre*, *Kalmia glauca*, *Oxycoccus oxycoccus*, and *Drosea rotundifolia* are among other characteristic species. The bogs occur surrounded by treeless areas, by tundras, or by coniferous forests, and vary much in area.—GEO. D. FULLER.

Ecological aspects of Paleozoic vegetation.—DACHNOWSKI[19] has given an account of the probable vegetational features and ecological conditions of Ohio from Ordovician through Pennsylvanian time. The most important part of this paper is the discussion relative to the prevailing xeromorphy of Paleozoic land plants. It has long been known that most of these xeromorphic

[16] BANCROFT, N., Note on vegetative reproduction in some Indian selaginellas. Ann. Botany 28:685–693. *pl. 49. figs. 7.* 1914.

[17] MARSH, A. S., The anatomy of some xerophilous species of *Cheilanthes* and *Pellaea*. Ann. Botany 28:671–684. *figs. 11.* 1914.

[18] RIGG, G. B., Notes on the flora of some Alaskan sphagnum bogs. Plant World 17:176–183. 1914.

[19] DACHNOWSKI, A., The ancient vegetation of Ohio and its ecological conditions for growth. Ohio Naturalist 11:312–331. 1911; Amer. Jour. Sci. 32:33–39. 1911.

plants were inhabitants of swamps, and it is the author's belief that the toxic theory, which he has done so much to develop, explains these ancient xerophytic structures as well as it does the xerophytic structures of modern bog plants.—H. C. Cowles.

Seedling anatomy.—Miss Thomas[20] has added a large body of facts to our knowledge of seedling anatomy, having investigated 150 species belonging to Ranales, Rhoeadales, and Rosales, about half of them belonging to Ranales. She has reached some interesting conclusions as to the phylogenetic relations of the various anatomical conditions, and is inclined to believe that seedling anatomy may be of service in indicating relationships, in spite of the recent tendency to discount it. It would be of interest if Miss Thomas should "summarize or analyze" the results obtained thus far, and give us a profitable perspective.—J. M. C.

Scinaia.—Setchell[21] has studied the species of red algae which have usually passed for *Scinaia*. As a result he has broken up what seems to be a plexus of forms. After a description of the morphology of the group, the taxonomic presentation includes *Scinaia*, with 11 species, 5 of which are new; *Gloiophloea*, with 7 species, 4 of which are new; and *Pseudoscinaia*, a new genus with two species. The discussion of geographical distribution of this group of forms is particularly suggestive, a subject to which the author has been giving much attention.—J. M. C.

Mutation in Egyptian cotton.—Kearney[22] has contributed to the literature of mutation by describing the behavior of Egyptian cotton, which exhibits the tendency characterizing *Oenothera Lamarckiana*, new characters appearing at different times and in different places. The origin of this cotton is obscure, but it seems certain that the varieties now grown are of mixed ancestry. If this be true, it would confirm the view that the tendency to produce mutants is a result of remote or complex hybridization.—J. M. C.

Elementary species of Onagra.—Bartlett[23] has published 12 new elementary species of the subgenus *Onagra*, 5 of them belonging to the aggregate called *O. biennis* in our manuals, 2 of them being allies of *O. parviflora*, and the remaining 5 being included in the recent descriptions of *O. muricata*, which in

[20] Thomas, E. N., Seedling anatomy of Ranales, Rhoeadales, and Rosales. Ann. Botany 28:695–733. *pls. 50, 51. figs. 43.* 1914.

[21] Setchell, W. A., The *Scinaia* assemblage. Univ. Calif. Publ. Bot. 6:79–152. *pls. 10–16.* 1914.

[22] Kearney, Thomas H., Mutation in Egyptian cotton. Jour. Agric. Research 2:287–302. *pls. 17–25.* 1914.

[23] Bartlett, H. H., Twelve elementary species of *Onagra*. Cybele Columbiana 1:37–56. *pls. 1–5.* 1914.

turn was referred to *O. biennis* until recently. These numerous elementary species emphasize the fact that *O. biennis*, as formerly understood, was really a surprising mixture of forms.—J. M. C.

Bolivian plants.—The Bolivian collections of TH. HERZOG, made during 1910 and 1911, have been determined by various specialists, and the first part is now published,[24] containing 339 species distributed as follows: ferns 136, Lycopodiales 1 (*Isoetes*), gymnosperms 2 (*Ephedra*), dicotyledons 190, and monocotyledons 10. The new species number 42, of which 14 are ferns, distributed among 10 genera, and 28 are dicotyledons, distributed among 17 genera.—J. M. C.

Lepidopteris and Antholithus.—ANTEVS[25] has made a critical investigation of *Lepidopteris Ottonis* and *Antholithus Zeilleri* and has concluded that they belong to the same plant. The latter is presumed to be the staminate structure of a seed plant; but no seeds are available to indicate whether this mesozoic plant represents a continuation of the Cycadofilicales, as the foliage would suggest, or is a member of some more modern group of seed plants.—J. M. C.

New genus of Plasmodiophoraceae.—FERDINANDSEN and WINGE[26] have described a new genus (*Ostenfeldiella*) of the Plasmodiophoraceae, dedicating it to its discoverer, Dr. C. H. OSTENFELD, who found it attacking a species of *Diplanthera*, growing in shallow water or muddy soil on the coast of the island of St. Croix. The parasite causes swellings in certain branches, so that the branch as a whole bears "a certain resemblance to a string of pearls."—J. M. C.

The vegetation of Guiana and Trinidad.—CAMPBELL[27] has given an interesting account of a visit to Guiana and Trinidad. Both British and Dutch Guiana were included in the itinerary. In each of these colonies and also in Trinidad study was facilitated by botanical gardens. Excellent stretches of natural forest are available from Paramaribo and Port of Spain.—H. C. COWLES.

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[24] (HERZOG, TH.), Die von Dr. TH. HERZOG auf seiner zweiten Reise durch Bolivien in den Jahren 1910 und 1911 gesammelten Pflanzen. Teil I. Mededeel. Van Rijks Herb. no. 19. pp. 84. 1913.

[25] ANTEVS, ERNST, *Lepidopteris Ottonis* (Göpp.) Schimp. and *Antholithus Zeilleri* Nath. Kgl. Svensk. Vetensk. Handl. **51**: no. 7. pp. 18. *pls. 3.* 1914.

[26] FERDINANDSEN, C., and WINGE, O., *Ostenfeldiella*, a new genus of Plasmodiophoraceae. Ann. Botany **28**:643–649. *pl. 45. figs. 4.* 1914.

[27] CAMPBELL, D. H., Some impressions of the flora of Guiana and Trinidad. Pop. Sci. Monthly **82**:19–32. *figs. 3.* 1913.

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THE ALPINE AND SUBALPINE VEGETATION OF THE LAKE TAHOE REGION

F. J. SMILEY

(WITH FOUR FIGURES)

Geology

The country rock of the region is mainly a coarse granite, which represents the ancient Sierran batholith. The sedimentary rocks which formed the more ancient surface, and under which the intrusives were thrust, have been for the most part completely eroded away; what little remains, as on the summit of Mt. Tallac, has been changed by pressure and heat into schistose rocks, and these have become deeply fissured by jointing. Under the degrading influence of the alpine climate, with rapid changes of temperature and moisture, these jointed slates and schists have been ever more deeply fractured and loosened from place, giving rise to the immense heaps of angular talus, which skirt the bases of Mt. Tallac, Maggie's Peaks, and Castle Peak. The metamorphosed sedimentary rocks increase southward, but REID (1) points out that "The largest areas are little more than a veneer over the granite, so that it is evident that the work of removing the roof of the granite is nearly complete."

With the granite the course of events has been in part similar, in part quite different, depending upon the amount of jointing. Where the granite is deeply fissured, the rock is quickly broken to fragments of varying size, and these, falling from place, are soon reduced to coarse sand, often deeply colored with iron. Where the

joints are horizontal the peaks have a terraced appearance, as on the south flank of Pyramid Peak. The sandy slopes formed from the crumbling granite are very pervious to water, and on their higher reaches only such plants as have especial advantages in obtaining a requisite amount of water can survive. At their bases one finds a distinctly mesophytic association, for the water absorbed above oozes out below and may be of amount sufficient to cause marsh conditions. On the east side of Angora Peak is such a sand slope whereon the plants become progressively more mesophytic as the foot is neared. Since the soil is the same from top to bottom, except for the increasing amount of organic material present, such a slope seems to be a direct, though imperfect, gauge for the water factor in plant life.

Where the granite is massive the process of rock decay is entirely different. While the factors concerned (temperature and moisture) are the same, their action is largely neutralized by the lack of rock fissuring; rock decay becomes almost entirely a matter of exfoliation. Huge slabs become broken from the surface and by their position protect the rock beneath; only as the slabs are slowly broken and slide from place can the process continue. In the granite deserts of Desolation Valley, Rubicon Valley, and Donner Pass, this protecting action of the exfoliated slabs may be particularly well seen. Here the only spots capable of bearing a flora are the small depressions between the glaciated ridges with soil formed in large part of wind blown granite dust. Over the greater surface plant life is impossible save for crustose lichens and a few hardy crevice plants, which have settled in the fissures about the borders of the slabs.

Limestone seems to be lacking in the district, though so abundant and important a rock base of the Basin ranges.

The rocks derived from extrusive lavas are mainly andesites and basalts. The principal vents, from which these flows issued, lie on the crest of the so-called Great Western Divide. From Round Top Peak, some 30 miles south of Lake Tahoe, there is a succession of ancient volcanoes terminating in Castle Peak, northwest of Truckee. The chief center for these flows seems to have been Mt. Mildred (2), 10 miles west of Lake Tahoe. These irruptives

now commonly lie on the top of ridges overlying the schists and granites. On decay they produce a dark red soil, over which are scattered angular or roughly rounded gray or brown andesite boulders.

The basalts, which locally may be present in considerable amount, lie above the andesite, which frequently shows a metamorphosed condition due to the heat and pressure; on the summit of Mt. Tallac is such a contact. These basaltic irruptives are the

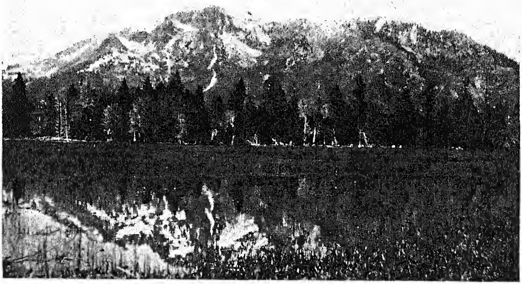

FIG. 1.—Transition zone: pond vegetation with climax forest; Mount Tallac in the distance.

most recent general feature of the geology, and the time intervening to the present has not been sufficient to materially modify the flows.

The whole district, except the highest summits, has been subjected to severe glaciation. This occurred in the later part of the Pleistocene, and the interval since has been too short to obscure the evidences of ice action, particularly at the higher levels where the surface was swept bare. Today these barren areas lie exposed, practically destitute of soil, and constitute some of the most striking evidences of glacial action to be found in the Sierra. At the time of maximum ice the higher levels were all filled with névé

fields which fed the glaciers moving down the valleys, transporting the débris that now forms the morainal heaps at 6000–7000 feet. The peaks and ridges, left after the débris was carried away, show many typical features of glaciated mountains: cirques, aretes, dents. A most perfect example of the last is Pyramid Peak, on whose north slope the Rubicon Glacier had its source, thence moving north, scouring out Rubicon Valley and probably debouching into Lake Tahoe through Rubicon Pass (7150 feet) and the valley of McKinney Creek.

Ice action varied in its effect upon the relief with the several rocks beneath the surface of the glaciers; in the andesite the glacial sapping is especially marked and the cirques of Twin Peak and Mt. Tallac are excellent examples of their kind. On the massive granite the effect seems to have been practically limited to clearing away the loose material overlying the bed rock, which was little, if at all, affected.

Topography

The region studied is embraced within the area mapped by the United States Geological Survey on the Truckee and Pyramid Peak quadrangles. It is roughly some 50 miles long and 15 wide, and may be considered as a typical section of the Central Sierra. This section of the range does not contain elevations comparable to those further south in the High Sierra of Fresno and Tulare counties, nevertheless several of the peaks rise above 10,000 feet, and one, Freel's Peak, is over 11,000 feet. The area is in shape a trough, with average elevation of the floor about 6500 feet. The rim of the trough is formed on the west of the Great Western Divide, which separates those streams flowing westward to the Great Valley of California from those forming a part of the Great Basin drainage system. The eastern margin is the Carson Range, the most eastern of the Sierran ranges.

The Great Western Divide is the more deeply dissected of these limiting mountain chains. From Round Top (10,430 feet) in Alpine County this dividing ridge runs north to Echo Lake and then bears west of north, continuing beyond Mt. Lola (9167 feet) into Sierra County. Along this height of land the more promi-

nent peaks are Mt. Tallac (9785 feet), Rubicon Peak (9193 feet), Twin Peak (8924 feet), Tinker Knob (9020 feet), and Castle Peak (9139 feet). The average height of the ridge is above 8000 feet. To the west it falls by a gradual slope to valleys draining into the Yuba, American, and Cosumnes rivers. Its eastern side forms an abrupt scarp closely skirting Lake Tahoe and the valley of the Truckee River. From the lake this scarp appears in places to be almost sheer. From the summit of Mt. Tallac there is a descent

FIG. 2.—Canadian zone: the high summit is Pyramid Peak

of over 3000 feet in less than two miles. There is some reason to think that fairly uniform elevation of the ridge really represents the remnant of an ancient plateau, traces of which still persist on the south side of Mt. Tallac and Angora Peak. It has been suggested (1) that the summits themselves represent a still older erosion surface, but for this there is little direct evidence. This summit accordance is a general feature of the Sierra, particularly marked in the High Sierra (3). On this ancient upland lie the high alpine valleys, of which Faith, Hope, and Charity valleys south of Lake Tahoe are typical.

The Carson Range, of which Freel's Peak is the highest summit, is less rugged than the Divide. Its summits are rounded or

even flattened cones, though the second highest peak, Mt. Rose (10,800 feet), has a steep ascent to the top. Its much more gentle contours are no doubt due to receiving far less rainfall than the Divide, and to the erosional force being consequently many times less.

Between these limiting ranges the drainage all centers in the Truckee River. The Upper Truckee rises north of Round Top, enters Lake Tahoe east of Tallac and, as the Truckee River, emerges from the northwest corner of the lake. Its course is north to the point of union with Donner Creek and then northeast through the Truckee Canon to the floodplains about Reno, Nevada. All of its important tributaries enter from the west, having their sources in the Divide's many lakelets.

These alpine and subalpine tarns are among the greatest charms of the region. They commonly fill the glacial cirques and often form a series of small basins from whose lowest margin the connecting brook cascades to the major stream. These pools are being gradually silted up both by sediment washed in from the adjacent slopes and by the vegetation fringing the banks; in time they become marshes and finally meadows, which in turn yields to the forest, for, as shown below, the forest is in this region of the Sierra the ultimate phase, since the elevation is not great enough to cause a cold timber line. On the broad ridge between Gilmore Lake and Suzy Lake above Glen Alpine is such a series of nearly filled basins, the largest of which is already converted into a marshy meadow, on whose margin a young growth of lodge pole pine has started.

As these lakes are mainly in glacial basins, they are frequently banked on the low side by moraines, which in places become of major importance in the local topography. The glaciers of the region have formed large deposits as lateral and terminal moraines about Independence and Donner Lakes, and on the west shore of Lake Tahoe an ancient extension of the last has been cut off by several terminal moraines of the Fallen Leaf Glacier and now persists as a separate lake, three miles long. The moraines on the sides of this lake are especially large, the eastern one being as long as the lake and 900 feet high.

Some of the valleys through which the larger glaciers moved have the U-shaped cross-section characteristic of glaciated moun-

tain valleys, a form very different from the V-shaped canons on the west side of the Divide, and also differing from the lower reaches of Truckee River into which the ice seems not to have entered. A typical example of such a U-shaped valley is the depression once filled by the Fallen Leaf Glacier and now having for its center the channel of Glen Alpine Creek.

Climate

In attempting a sketch of climatic conditions in the high Sierra, one is confronted with the fact that exact observations are too few

Fig. 3.—Canadian and Hudsonian zones: Suzy Lake with Dick's Peak at right

to justify anything more than provisional statements. The data offered for the several stations have been gathered from the reports of the Weather Bureau; since detailed statement for the highest station has only been published since 1906, it has seemed best to make comparisons cover the same years even for stations where data of a kind is obtainable through a longer period.

The most constant feature of the alpine climate is the diminishing pressure with ascent. While there is little evidence to show that this factor is of itself important in the life of plants and animals, at least within the vertical range of the mountains of western North

America, yet it induces change in other climatological factors which are of great importance. "Le fait essentiel d'où dérivent à peu près tous les caractères du climat de montagne est la raréfaction de plus en plus grande de l'air dan les hautes altitudes. De tous les phénomènes météorologiques des régions élevées, c'est le plus régulier, car c'est le seul qui ne dépende pas des conditions locales du relief" (4). Among these changed factors may be mentioned increased insolation, increased radiation, change of illumination by increase of proportion of violet light, and rapid alteration from saturation to extreme dryness (5).

That any considerable ascent is accompanied by a fall of temperature is a constant phenomenon all the world over; it is due to the diminished heat capacity of the rarefied air. This fall in temperature may be counteracted within narrow limits by the relief; it is frequently noted in the mountains that plants which are indicators of a colder habitat are growing at a lower elevation than other plants commonly found much lower down. This is especially true in lately glaciated regions with their usually rugged topography; the cirques will often have a flora distinctly microthermic, while the surrounding ridge bears forms suggesting a milder climate. An example of such a contrast is afforded by the cirque on the northeast of Mt. Tallac, in which *Tsuga Mertensiana* is growing full 500 feet below *Pinus Jeffreyi* on the bluff overlooking Fallen Leaf Lake. This inversion of temperature, with the colder air sinking to the valleys and the warmer currents sweeping up the ridges, accounts in part for the lingering snow drifts that may lie in the cirques till late in the summer, or even persist throughout the season. It also produces a complexity in zonal maps, the limits of the warmer zones advancing up the slopes and the colder sinking below their average level.

In the higher Sierra low temperatures in winter are known comparable to those of the east. The lowest temperature reported from Tamarack, Alpine County (8000 feet), is -29°F. in January, 1910, or 51° of frost. On the summit of Mt. Rose the lowest record is -10° F. Summer temperatures may become fairly high; Tamarack reports 86° F. in July, and Summit, Placer County (7017 feet), 90° in October (table B). This last suggests a new feature

of the alpine climate: the displacing of the heat total toward the end of summer and fall. The start of the vegetative period (the local "spring") is delayed till summer is well advanced below, but the brilliant insolation of the alpine day in part compensates for this late beginning, so that "fall" phenomena are nearly contemporaneous both above and below. "Die Primeln blühen auf dem Rigi bei 1800 m. ca. 6 Wochen später als in Zürich, die Herbstzeitlose dagegen beinahe gleichzeitig" (6).

FIG. 4.—Hudsonian and Arctic-alpine zones: Desolation Valley at Lake LeConte and Pyramid Peak.

It has been stated that the zone of maximum rainfall in the Sierra is between 5000 and 7000 feet: Colfax (2421 feet), 46.64 in. per year; Cisco (5939 feet), 49.68 in. per year; Summit (7017 feet), 46.58 in. per year (mean of 30 years); but the diminished rainfall at Summit may be due to the influence of the arid east, an influence not barred by high ranges. A comparison of the same period (1909–13) shows at Summit (7017 feet) 40.98 in. mean total precipitation, and at Tamarack (8000 feet) 52.77 in. It will be noted that this would seem to have been a period of less than normal rainfall, the deviation amounting to 12.3 per cent. If such was the case, the precipitation at Tamarack should normally

be 59.24 in. On the eastern side of the Great Western Divide the precipitation falls rapidly, Truckee (5218 feet) having but 26.98 in. (table C).

Most of the annual precipitation in the high Sierra falls as snow and some astonishing totals are recorded from the Tahoe region. At Summit, in the winter of 1889–90, 776 in. of snow fell, or nearly 65 feet; in a record of 38 years the average was 417 inches (7). In the period limited by the winters of 1908–09 and 1911–12, the average annual snowfall at Tamarack was 515 in., at Emigrant Gap (5221 feet) 249.3 in., and at Glenbrook, Nevada, on the east shore of Lake Tahoe, 208.2 in. This last station, elevation 6282 feet, at the western base of the Carson Range, suggests the aridity of that range as compared to the Divide. At Summit on May 10 there is still a mean depth of snow of 20 inches, nor is the land fully cleared in average years till May 26. At Tamarack the end of June still sees the ground covered every other year. Severe frosts may occur at any time throughout the summer, but the rubric "killing frosts" of the weather reports is inapt when applied to the high mountains, for the simple reason that the plants may be frozen but are not killed. I saw on a morning in August plants of *Gentiana calycosa* stiff and brittle with frost on the shoulder of Mt. Tallac, yet in the afternoon the same colony was apparently none the worse for the freeze (table D).

This great burden of snow acts upon the plants of the district in at least three ways: it furthers tree growth, but impedes the growth of the forest; it favors the meadow, and particularly the wet meadow; it favors summer ephemerals.

In the Tahoe region at present there is no glacial ice, and but few snow banks persist for longer than one season. Where these occur, they are always on the east or northeast of the ridges and peaks, for the prevailing wind from the southwest piles up the snow on these exposures. No true snow line exists.

Alpine winds are keen and drying, and, in spite of their actually moving smaller masses of air, exert a marked influence upon the forms of plants. "Wind cripples" are a constant feature of the arboreal vegetation on the higher summits. On Mt. Rose velocities of 50 miles an hour have been recorded. In the spring a

"chinook" wind quite commonly melts the snow very rapidly. Beside these general winds, mention should be made of the so-called "mountain and valley" winds which reverse their movement twice daily, flowing down the valleys at nightfall and attaining considerable force when the valley narrows suddenly below, and flowing toward the summits when these have been heated by the morning sun. These winds are of moment in alpine plant distribution as being probably among the most effective agents for extending the vertical limits of species.

TABLE A

MEAN MONTHLY AND ANNUAL TEMPERATURE (F.)

	January	February	March	April	May	June	July	August	September	October	November	December	Annual
Truckee 5818 ft...	25.3	28.3	32.9	40.0	48.4	57.4	65.4	63.4	55.9	45.1	36.5	28.7	43.9
Summit 7017 ft...	26.8	27.7	32.0	35.9	44.6	51.2	57.6	58.0	53.8	46.5	34.8	28.0	41.4
Tamarack 8000 ft..	21.5	21.7	26.8	32.5	36.5	45.8	55.2	56.3	48.1	39.1	29.8	19.8	36.1

TABLE B

MONTHLY EXTREME TEMPERATURES*

	January			February			March			April			May			June		
Summit..	55	−4	35	52	−8	46	53	6	31	71	−2	30	85	12	39	82	28	43
Tamarack	49	−29	64	53	−25	64	64	−17	54	69	−14	58	81	6	46	80	18	50

	July			August			September			October			November			December		
Summit..	86	28	47	89	27	48	88	28	48	90	20	50	69	8	36	55	−5	28
Tamarack	86	24	47	82	30	48	80	20	50	76	2	49	62	−8	48	58	−26	52

* Under each month the first column gives the highest temperature ever recorded at the station; the second, the lowest temperature; and the third, greatest daily range ever recorded in the month.

TABLE C

TOTAL PRECIPITATION IN INCHES 1909–1913

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
Summit	13.58	3.22	6.22	2.69	1.17	0.07	0.97	0.03	1.0	0.85	4.94	6.24	40.98
Tamarack	16.85	5.08	4.74	3.39	1.78	2.40	1.5	0.31	1.12	1.75	6.69	7.16	52.77

TABLE D

SEASONABLE SNOWFALL IN INCHES

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	Year
Summit..........	T	6.2	38.7	52.2	166.2	47.2	62.0	23.5	12	408.0
Tamarack........	1.0	16.6	55.1	60.5	198.7	76.2	51.5	36.5	18	496.1

Life zones

TRANSITION ZONE.—The problem of attempting to discover the zonal limits of the various species collected in the Tahoe region is complicated by the very irregular topography; the line between adjacent zones is nowhere clearly defined, and, where the relief is especially rugged, often becomes very tortuous. Mapping can only express the general distribution, at least on maps of such a scale as are available. In addition to the complication caused by the relief and consequent frequent change of exposure, the soil characters cause a variableness in the zone boundaries; on the dark chocolate colored trap lives a flora whose members are distinctly more xerophytic than those of granite soils. A very evident change of plant life, for which this edaphic factor seems the only one assignable, is that found on the ridge connecting Suzy Lake with the mountain group culminating in Dick's Peak. The ridge runs northwest to southeast and the south slope is fairly even, but where the trail runs out of the granite into the trap a break in the general aspect of the flora occurs: typical Upper Transition on the granite with *Abies concolor* and *Pinus Jeffreyi* as the chief trees, and Canadian on the trap with *Juniperus occidentalis* giving the tone to the forest.

Another more difficult factor in the problem of assigning plants to definite zones is the fact that the plants often refuse to be so assigned; the stragglers from the general rank are too numerous. Yet in spite of this it seems desirable and feasible to group the vegetation within certain altitudinal categories and, while many individuals of a given species will be often found outside the zone of their greatest frequency, as a whole the assemblage of plants denominated the "Transition flora of the Sierra" has a general coherence, and the expression conveys a definite meaning to those who have considered the whole Sierran flora.

In the Tahoe region this Transition flora covers approximately 25 per cent of the total area, which is equivalent to saying that by far the larger part of that flora lies below the region considered in the present report, and we are here dealing with that fraction of the total which has been called Upper Transition, a flora showing more relationship to the life zones above than to those below. It is this fact of alliance with strictly alpine and subalpine floras that requires us to consider it here.

The Upper Transition forest, as of all the higher zones, is exclusively a coniferous forest; what few arboreal or arborescent angiosperms are present are only found close to streams or lake shores and hence are to be regarded as members of the stream bank association. This Transition coniferous forest is formed by *Abies concolor*, *Pinus Jeffreyi*, *Pinus ponderosa*, *Libocedrus decurrens*, and *Pinus Lambertiana*, in frequency in approximately the order named. The forest growth is dense in but a few favored localities, as on the floor of the ancient Fallen Leaf Glacier at the south end of Fallen Leaf Lake. Generally the trees are scattering and individual trees relatively small compared to the average size of the same species on the western slope of the Sierras. The white fir alone maintains its average form. The sugar pine is scarce, since in the district the moderately moist rich flats frequented by this tree commonly lie above its range. The yellow pine is a common tree about Tahoe and northward to Truckee, but is dwarfed in size, and appears excessively parasitized by *Arceuthobium occidentale*. At the south end of the lake the nearly allied species *Pinus Jeffreyi* makes with *Abies concolor* two-thirds of the forest cover. As a rule, the woods away from the streams are free from underbrush, the surface vegetation consisting of low perennials mainly, such as *Corallorhiza Bigelovii*, *Pyrola asarifolia incarnata*, *Pterospora Andromeda*, *Pedicularis semibarbata*, *Antennaria argentea*, *Antennaria Geyeri*, and in sunny glades *Balsamorhiza sagittata*, *Erigeron divergens*, and *Madia exigua*.

Next to the forest in importance in the general aspect of the Transition flora comes the stream bank association, which advances up all the water courses, cutting the coniferous forest into isolated fragments. In this stream bank vegetation *Populus trichocarpa*, *Populus tremuloides* (there is a charming aspen grove on the low

shelf just east of Fallen Leaf Lake), and *Salix lasiandra* form the superior stratum, and beneath them grow *Alnus tenuifolia*, *Rubus parviflorus*, and *Cornus pubescens* as undershrubs, while the herbs most frequently found are *Carex rostrata*, *Allium validum*, *Habenaria leucostachys*, *Thalictrum sparsiflorum*, *Cicuta vagans*, and *Heracleum lanatum*. The lowest stratum, made up of delicate herbs for the most part, shows *Listera convallarioides*, *Polygonum Douglasii*, *Kelloggia galioides* (which is also found in the forest), *Galium bifolium*, and *Anaphalis margaritacea*.

Scrub (chaparral) becomes of considerable importance on dry sunny slopes in the Tahoe Upper Transition; the principal species are *Amelanchier alnifolia*, *Cercocarpus ledifolius*, and on sandy benches *Artemisia tridentata*, the last being present as a considerable factor in all the zones below the true alpine. Among these shrubs one finds *Lilium Washingtonianum*, *Eriogonum nudum*, *Collomia tinctoria*, *Mimulus Breweri*, *Mimulus leptaleus*, and *Mimulus Torreyi*.

When the relief becomes too sharp and the soil covering too scanty for chaparral shrubs, there is found a flora of rocky outcrops and benches, composed of small perennials and annuals. Here grow *Eriogonum Douglasii*, *Oxytheca spergulina*, *Heuchera rubescens lithophila*, *Apocynum androsaemifolium*, *Gilia pungens*, *Cryptanthe affinis*, a form of *Monardella odoratissima* (*Madronella pallida* Heller), *Adenostegia tenuis*, *Chrysopsis hispida*, and *Sericocarpus rigidus*.

The meadow formation is well developed along Truckee River, about Donner Lake, and where the Upper Truckee enters Lake Tahoe both east and west of Tallac. Almost exclusively it is a wet meadow, since the dry meadow is speedily invaded by trees and grows into the climax forest. Such an invasion and young forest growth is now taking place just back of Tallac, *Pinus Jeffreyi* seedlings being the chief entrants. On the wet meadow are *Sparganium simplex*, many carices (*Carex lanuginosa* here below its average level, *Carex nebraskensis*), *Juncus nevadensis*, *Veratrum californicum*, *Urtica gracilis*, *Delphinium decorum patens*, *Trifolium cyathiferum*, with *Trifolium pratense* and *Trifolium repens* as immigrants into the meadows about Donner Lake, *Hypericum*

Scouleri, *Agastache urticifolia*, and *Solidago elongata*, the two last being especially common about meadow borders within the ring of *Salix macrocarpa argentea* that commonly hedges the wet meadow. The dry meadow formation, as stated above, is less evident, but still exists and shows a considerable list of species: *Sporobolus depauperatus*, *Zygadenus venenosus*, *Myosurus apetalus*, *Hosackia americana*, *H. crassifolia*, *Gomphocarpus cordifolius*, *Allocarya hispidula*, *Cryptanthe geminata*, and *Aster canescens* being the more numerous.

Canadian zone.—Of the several life zones discoverable in the Tahoe flora, the Canadian is at once the most extensive and most difficult to define. Its lower limit conforms generally to the 7000 feet contour line, while the upper boundary may be placed at about 8500 feet as a maximum; within this range of 1500 feet lies the greater part of the district. This zone includes most of the ridges connecting the peaks rising into the alpine region; it covers the lower flank of the Divide, and encircles Mt. Rose below 9000 feet, for in the Carson Range the greater aridity and higher mean temperature of the growing season causes all the life zones to rise higher than they do in the mountains west of Lake Tahoe. This rise of the zonal limits reaches a maximum on Freel's Peak, which has small groves of *Pinus Murrayana*, perhaps the one best "Leitpflanze" of the Canadian flora, even at the 10,000 feet level. These slopes are frequently composed of loose granite sand, and, as mentioned in the case of the slope east of Angora Peak, support a characteristic flora of shrubs rarely found outside the Canadian life zone. The edaphic factor must be constantly kept in mind in locating zonal limits, and in our district this is largely conditioned by the degree of glaciation and subsequent erosion. Where the country rock was swept bare, a typical Hudsonian assemblage of plants is apt to be found, even though well below the 8500 feet limit, as in Desolation Valley at 7800–8000 feet. Where deposition has given a sufficient soil cover, conditions are decidedly ameliorated, and the Canadian flora develops typically even above its general level, as in the high alpine valleys south of Lake Tahoe.

The Canadian forest is, for the most part, a thin forest; as a rule the trees stand well apart, only exceptionally preventing

sunlight reaching the ground in quantity insufficient for a varied herbaceous ground flora. This in effect brings the meadow into the forest, or rather the forest into the meadow, since the last is antecedent to the climax vegetation of the district, the coniferous forest. There is then difficulty in describing these associations apart, though they are distinct enough below.

The one tree of this forest which usually forms dense stands is the red fir (*Abies magnifica*). It favors low benches and bottoms of valleys and is not often found upon the slopes save as a fringing forest along water courses. It does form a considerable element in the forest on the great moraines, however, the loosely aggregated soil of which permits deep root penetration. On the great moraine east of Fallen Leaf Lake the red fir is the principal tree. This dark fir forest has few shrubs, but does support a characteristic flora of ericaceous perennials, such as *Pyrola pallida*, *P. picta*, *Chimaphila Menziesii*, *C. umbellata*, and *Sarcodes sanguinea*. The fir forest is an exclusive association, few of the other Canadian species entering into it, doubtless excluded by the insufficient light for seedlings.

The Canadian pine forest has a very different aspect, being open or even parklike in the spacing of the trees. Neither of the two pines which compose it (*Pinus Murrayana* and *Pinus monticola*) attain large size, but are widely branching, especially at their upper levels. *Pinus monticola* continues into the Hudsonian and at times becomes a tree line form, but *Pinus Murrayana* is relatively constant about Lake Tahoe as a Canadian exemplar. The lodge pole pine is a vigorous seeder, and all about the meadows in the Canadian, where drainage has permitted, the young seedlings form a dense border. By subsequent drying out of the weaker individuals the open character of the mature forest is attained. *Pinus Murrayana* is often attacked by *Arceuthobium americanum*.

Within this open forest grow several shrubs: *Salix Scouleriana* along the damp ravines, and with it *Vaccinium occidentale*, *Ribes cereum*, *R. nevadense*, *Purshia tridentata* (this forming rounded clumps which in the Hudsonian become dense polsters), and above all as a typical undershrub, *Ceanothus cordulatus*. The herbaceous flora embraces *Melica aristata*, *Spraguea umbellata*, *Stellaria Jamesiana*, *Lupinus calcaratus*, *L. apertus*, *Viola Nuttallii*, *Ortho-*

carpus cryptanthus, *Aster integrifolius*, *A. yosemitanus*, *Erigeron inornatus*, and *Senecio lugens*.

The other tree found commonly in the Canadian is the Sierra juniper (*J. occidentalis* Hook), which in the Tahoe region is confined exclusively, so far as I could determine, to the slate outcrops, never appearing on granite in this lowest of the boreal zones. It often attains large girth at base (one measured on the Dick's Peak-Suzy Lake trail was 16 feet in circumference), but branches low and reaches a height of 20–25 feet. At Camp Agassiz, at the lower limit of the Canadian, it is parasitized by *Phoradendron juniperinum*.

The Canadian scrub (chaparral) is a constant feature of the vegetation on the dry rocky hillsides: *Amelanchier alnifolia* and *Cercocarpus ledifolius* continue up from the Transition and are associated with *Amelanchier glabra*, *Holodiscus microphyllus*, *Ceanothus velutinus*, *Arctostaphylos patula*, *A. nevadensis*, and *Grossularia Roezli*. On these dry slopes among the shrubs are found *Silene Douglasii*, *Gayophytum ramosissimum*, *Zauschneria californica*, *Chaenactis Douglasii*, and *Eupatorium occidentale*. On the slopes where the weathering has reduced the rock débris to finer particles we find at the top sedums deeply rooted in the loose sand (*Sedum obtusatum* and its close ally *Gormania Burnhami*), and below, where the influence of the water content of the slope becomes appreciable, bordering thickets of *Acer glabrum* (*Acer Torreyi* Greene), *Sorbus californica*, and below these, *Prunus emarginata*. *Spiraea arbuscula* occurs at the base of such slopes where the water supply is abundant.

The true rock plants include *Quercus vaccinifolia*, the only oak of the boreal region, which forms dense espaliers over rounded rock surfaces, but is more evident as a cover for the roches moutonnées in the glaciated Hudsonian valleys. This shrub seems to be restricted to the granite at Lake Tahoe. *Nama Lobbii* is a plant of similar habit and covers many granite boulders about Cisco. Other rock-plants are *Eriogonum Lobii*, *E. umbellatum*, *Sedum stenopetalum* (the last forming extensive patches), *Lupinus Breweri* (a typical dry lithophyte), *Apocynum androsaemifolium pumilum*, *Pentstemon Jaffrayanus*, *P. deustus*, and *Hieracium horridum*. The xerophytic ferns *Pellaea Breweri* and *P. Bridgesii* are common on the trap outcrops, while another rock fern of similar habit, *Woodsia scopulina*, was seen but once.

The series of hydrophytic associations begins with plants of lakes and pools. Of these in the Tahoe Canadian are present about the edge of the basins *Nymphaea polysepalum*, *Callitriche verna*, *Hippuris vulgaris* (very abundant in Lily Lake near Glen Alpine), and the buckbean (*Menyanthes trifoliata*). These plants of the open waters in decaying advance the margin of the land into the water; in their wake appears *Carex spectabilis*. The drying margin supports a growth of willows (*Salix californica*, *S. Lemmonii*, and at the higher levels *S. sitchensis*), while beneath them grow *Ranunculus flammula reptans*, *Cheiranthes asper*, and in such an evironment in one locality was found *Botrychium californicum*.

The ultimate end of such an invasion is the filling of the lake and beginning of the wet meadow association, which about Lake Tahoe in the subalpine includes *Sparganium simplex*, *Sagittaria latifolia*, *Carex aurea*, *Juncus bufonius*, *Veratrum californicum*, *Spiranthes Romanzoffiana*, *Ranunculus alismaefolius alismellus* (continues into Hudsonian and alpine), *Hosackia Torreyi*, *Hypericum anagalloides*, *Veronica humifusa*, *Helenium Bigelovii*, and allied forms.

On the drier edge of such swampy meadows are found *Agropyron divergens*, *Phleum alpinum*, *Stipa occidentalis*, *Tofieldia intermedia*, *Polygonum aviculare*, *Saxifraga integrifolia sierrae*, *Frasera speciosa*, *Pedicularis attolens*, *Arnica mollis*, and *Erigeron salsuginosus*, the last being more abundant in similar localities in the Hudsonian. About the edge of such meadows and along their drainage channels will be found *Salix macrocarpa argentea*, *Cornus pubescens*, and *Alnus tenuifolia*, the last a characteristic Canadian shrub of stream banks.

When the drainage has progressed beyond the wet meadow stage, such plants as *Melica fugax*, *Phleum pratense*, *Stipa viridula*, *Allium campanulatum*, *Calochortus Leichtlinii*, *Polygonum imbricatum*, *P. Kelloggii*, *Lupinus sellulus*, *Epilobium brevistylum*, *Gilia Harknessi*, *G. ciliata*, *Erigeron Breweri*, and *Gnaphalium palustre* appear to be followed by seedlings of the lodge pole pine and the ultimate forest phase.

The third distinctive association, that of the fringing vegetation of stream channels, includes such plants as *Habenaria sparsiflora*, *Aconitum columbianum*, *Aquilegia truncata*, *Delphinium glaucum*,

Sphenosciadium capitellatum, besides the willows and the alder above named.

HUDSONIAN ZONE.—This zone in the generally accepted scheme of zonal arrangement ends at tree line; it ought then by hypothesis to be a simple matter to distinguish the upper limit. Unfortunately, tree line is conditioned by so many factors, any one of which may be decisive at any particular place, that in the field it is often nearly impossible to define the line that separates the last of the forest zones from the true alps above. In the Tahoe district this is all the more true since, as stated above, a *cold* tree line seems not to exist. The factors that impede and ultimately prevent tree growth are in our district wind currents, edaphic conditions, and the mechanical effect of deep snow.

The formative influence of wind upon tree growth is apparent enough at low levels; trees near a beach are commonly strongly modified in shape, but in this case the mechanical effect of the wind seems to account for much of what is observed. At high latitudes and on high mountains the wind appears to exert the same stress plus a drying out power which the tree cannot withstand. In the Tahoe region it is this desiccating wind that most often determines the limit to the forest; on one side of an arête tree growth will dwindle out scores, even hundreds of feet below the summit, while on the other side trees of normal shape rise to the top. As the wind is prevailingly from the southwest, trees on a north slope ought to rise higher, other conditions being equal. This they do in a few cases, as on the north side of the arête above Gilmore Lake, connecting Mt. Tallac and Jack's Peak. More often the sheltering effect of the ridge is discounted by the deep snow drifts which the southwest wind drops on the north and east sides of the peaks and ridges. In the glacial cirques on the east side of the Divide one commonly finds trees growing up to the chord of the arc, rarely within the cirque itself, and the inference seems warranted that this tree line is a deep snow line. Quite as often the tree line is a product of the soil conditions; trees cannot grow on the unfractured rock, and where this is massive and exposed a tree line exists; this is the explanation of the treeless Rubicon Valley. A limit to the growth of trees is also set by an excess of ground water; many of the peaks and ridges about Lake Tahoe

are unwooded at their summits because of wind conditions; their sides are compassed by heaps of talus more or less fragmented, but not affording a footing for the seedling pine or fir, while below these rubble heaps marsh conditions prevail due to the seepage of water from the slopes, and the trees are kept at a distance. All of these factors, either singly or combined, operate to make the line a very sinuous one that divides the true alpine region from the Hudsonian forest.

In that forest the principal trees are the white bark pine (*P. albicaulis*), the silver pine (*P. monticola*), the Sierra juniper, and the alpine hemlock (*Tsuga mertensiana*). The first is the tree line tree of the Sierras par excellence, being found along the whole Sierran crest. In the Tahoe region the largest forest of this pine is on the southwest flank of Mt. Tallac; here it forms a nearly pure stand and decreases from a tree 40–50 feet in height to prostrate wind cripples at the base of the actual peak. The finest examples of single trees noted grew on the plateau between Angora Peak and Ralston's Peak. *Pinus monticola* is not common in the south end of the Tahoe district, but increases northward about Cisco. The Sierra juniper (*J. occidentalis*) appears to have an interesting distribution about Tahoe; in the Canadian it is always found on the slate outcrops, but in the Hudsonian seems to be the chief krummholz tree of granite basins. It was not noted on the higher lying volcanics (andesites). The chief groves of the alpine hemlocks in the region are on the Lucile Ridge and in Desolation Valley, where along the east side of the valley it forms a pretty continuous forest for several miles. Unlike the forest of white bark and silver pines, the hemlock forest is dark and the ground flora sparse. In the more open pine forest the ground cover is made up in part of *Polygonum Davisiae*, *Fragaria virginiana platypetala* forma *sibbaldifolia* Hall, *Lupinus Lobbii*, *L. meionanthus*, *L. montigenus*, *Epilobium obcordatum* (the last on the higher ridges), *Hieracium gracile detonsum*, and *Whitneya dealbata*. About the edges of the hemlock forest were growing *Aster Andersoni* and *Artemisia norvegica*.

Above the forest, rising toward the peaks and arêtes, are extensive talus slopes, and higher still the country rock offers on ledges and in crevices lodgement to many plants of peculiar habit and extraordinary adaptation to their inhospitable surroundings. It is

on this rocky surface that one finds many of the growth forms associated with extreme life conditions: polsters, mat plants, espaliers. It is this region of the high mountains that offers the closest analogies to the forms of desert plants, and it is here that some of the peculiarly desert genera (such as *Eriogonum* and *Artemisia* among flowering plants, *Cheilanthes* for desert ferns) have made their deepest mark upon the alpine flora. Conditions on the broken talus are quite different from those of ledges, and a different group of plants grows on it: a high altitude dwarf chaparral formed of *Ribes montigenum*, *R. viscossissimum Hallii*, *Grossularia lasiantha*, *Purshia tridentata*, *Aplopappus macronema*, and *Artemisia arbuscula*. These may all grow intricately together, or separately, when all assume the same growth form, hemispherical polsters. On the more solid rock the mat form is the more common (*Spraguea umbellatata*, *Phlox Douglasii diffusa*, *Chaenactis nevadensis*). In crevices will be found *Eriogonum marifolium*, *E. Wrightii*, *Gilia congesta palmifrons*, *Polemonium pulcherrimum*, *Eriophyllum integrifolium*, and *Senecio canus*. Over the glaciated granite surfaces exposed in Desolation Valley *Quercus vaccinifolia* forms dense espaliers. On the granite also was found the only specimen seen of the arctic-alpine shrubby cinquefoil, *Potentilla fruticosa*. On wet granite ledges grow *Scirpus criniger*, *Sedum integrifolium*, *Parnassia californica*, and *Erigeron Coulteri*. Where a soil cover has accumulated on moist ridges are to be found *Cassiope Mertensiana*, *Lappula Cusickii*, and *Pentstemon procerus geniculatus*.

As in the Canadian, so in the Hudsonian pine forest the meadow spreads under the trees and among the grasses (*Agrostis Rossae*, *Festuca scabrella*, *Melica stricta*) will be growing *Brodiaea gracilis*, *Calochortus Leichtlinii*, *Gayophytum caesium*, and *Aplopappus apargioides*. A characteristic feature of high mountain meadows is the large percentage of carices and junci present; in the Hudsonian meadows of Lake Tahoe have been identified *Carex capitata*, *C. Helleri*, *C. luzulaefolia*, *Scirpus pauciflorus*, *Juncus Parryi*, *J. nevadensis*, and *J. subtriflorus*. Common herbs of these wet meadows are *Stellaria longipes*, *Saxifraga bryophora*, *Trifolium monanthum*, *Gentiana Newberryi*, *Mimulus pilosellus*, *Antennaria media*, and *Erigeron salsuginosus*.

Hudsonian lakes are generally fringed by *Salix glaucops*, *Ledum glandulosum*, and *Phyllodoce Breweri;* while along the marshy margins of the outlet will be found *Carex straminiformis*, *Viola Macloskeyi*, *Dodecatheon alpinum nanum*, and *Kalmia glauca microphylla*.

ARCTIC-ALPINE.—A feature of the flora of the boreal region as compared to the Transition is the gradual merging of the forest and meadow due to the thinning out of the former. At tree line the forest becomes zero and the meadow becomes the dominant formation, for in a sense the rocky fields about the summits themselves are inchoate meadows; between the boulders meadow conditions prevail. These diminutive meadows do not differ essentially from the larger expanses below the rock fields. The only formations to dispute the territory are that of rock crevices and the true lithophytes.

As stated above, it is questionable if a true climatic tree line exists about Lake Tahoe; nevertheless certain plants (*Oxyria digyna*, *Ranunculus oxynotus*, *Draba glacialis*, *Juniperus communis sibirica*, *Primula suffrutescens*, *Hulsea algida*, *Ivesia Schockleyi* may be cited) are present, which are constantly found only above tree line in regions where such a line unquestionably exists. In the absence of a better understanding of this vestigial arctic-alpine flora, a discussion at present would be premature.

STANFORD UNIVERSITY

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ON THE MALE GAMETOPHYTE OF PICEA CANADENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 200

A. H. HUTCHINSON

(WITH PLATES XV–XIX AND ONE FIGURE)

The male gametophyte of *Picea excelsa* has been described by STRASBURGER, MIYAKE, and POLLOCK. At the shedding stage, as recorded by STRASBURGER (1), there are two disintegrating prothallial cells, a stalk cell, a body cell, and a tube nucleus. MIYAKE (2) verified this account; also described the pollen tube stages and the division of the antheridial cell into stalk and body cells. POLLOCK (3) noted certain variations in the gametophyte at the time of pollination. This account deals with the early stages of development in the male gametophyte of *Picea canadensis*.

The staminate cones were collected from trees growing near Lake Simcoe, Ontario, Canada. Daily collections were made from May 2 until May 15, the time of shedding. The usual time for pollination in this locality is about two weeks later.

Nomenclature

The nomenclature used in accounts of male gametophytes has varied according to the character used as a basis for the system, whether it be size, position, or the writer's conception of origin or function of the different cells. Early in the nineteenth century FITZSCHE described *Pinus* as having a large central vesicle and disintegrating bodies against the wall of the pollen grain (*Zwischenkorper*). MEYEN (in 1839) stated that the *Zwischenkorper* were cells, and that one of them served as a stalk of attachment. JURANYI (4) reported that in *Ceratozamia* the pollen mother cell divided into a large and a small daughter cell (*kleine Tochterzelle*); that the latter divided to form two, and that the inner of these gave rise by division to an inner cell and an end cell. These three cells were collectively known as the cell body (*Zellkörper*). Until 1891 the tube nucleus (*grosse Zelle* or *freigebildete Zelle*) was believed

to be the fertilization nucleus. At that time BELAJEFF (6) showed that in *Taxus baccata* the larger cell is not the generative cell; but that the small cell divides in the tube and one of the derivatives becomes the generative cell.

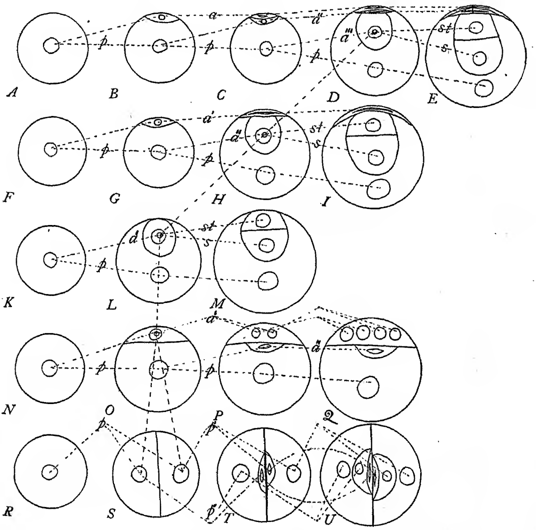
In 1892 STRASBURGER (1) described *Ginkgo biloba* as having two prothallial cells, representing a vegetative prothallus, and an antheridial cell, which are successively cut off from the pollen cell; the antheridial cell divides into stalk and body; the latter produces the sperms. The large pollen nucleus, because of its foremost position in the tube, was called the tube nucleus. The general conception of origin and function is the same today. It is not surprising, however, that, with such a variety of types, different investigators have since used different terms to designate similar cells.

The system of nomenclature to be used in this account has been made necessary by the nature of the gametophytic development. The primary cell (*P*) is regarded as retaining its identity, just as an apical cell. In the pollen tube stages it is represented by the tube nucleus. The successive divisions of the primary nucleus are known as primary divisions; the cells cut off are called, tentatively, the first, second, or third primary derivatives (a', a'', a''' in figs.). Divisions of the latter cells are called secondary. The cell which later divides to form male nuclei is termed spermatogenous (*s* in figs.); the sister non-functioning cell is the sterile cell (*st* in figs.). The mother cell of a spermatogenous and a sterile cell is called an antheridial cell.

Development of the male gametophyte

The first primary division is variable; the cell wall may cut off a lenticular polar cell (fig. 7); it may be oriented at right angles to the longitudinal axis, cutting off approximately one-third of the protoplasmic mass (figs. 6, 33, 37, 53); it may be in the plane of the vertical axis, in which case two nearly equal cells result (figs. 2, 10, 11, 13, 31, 32); it is often inclined (figs. 28, 29); and occasionally no dividing wall is formed, the two resulting nuclei being then free in the cytoplasmic mass (figs. 8, 9, 12). The further development of the resulting cells is largely determined by the

nature of the first primary division. When the primary derivative is small and lenticular, it rapidly degenerates (figs. A-I and 20–26 etc.); when the division is median or nearly so, each of the two cells formed has the power of repeated division, giving rise to a



FIGS. A–U.—A diagram to illustrate five types of development in male gametophytes of *Picea canadensis:* *P*, primary cell; *a'*, *a''*, *a'''*, the first, second, and third (potentially) antheridial cells; *s*, spermatogenous cell; *st*, sterile cell; the dotted lines indicate origin and sequence; relative size is also shown; figs. A–E, three primary divisions, first and second cells cut off evanescent, third by a secondary division produces a spermatogenous and a sterile cell; figs. F–I, two primary divisions, second cell cut off from primary cell functions as antheridial; figs. K–M, first primary derivative functions as antheridial cell; figs. N–Q, two primary divisions give rise to two antheridial cells, repeated divisions of the first produce four free nuclei; figs. R–U, primary cell divides to form sister primary cells, each of which produces an antheridial group, a bi-antheridial gametophyte.

so-called double gametophyte (figs. R–U and 28, 29, 31, 32); when the first primary derivative is surrounded by cytoplasm it divides, giving rise to a spermatogenous and a sterile cell (figs. K–M and 9, 12, 30). In this case there is no further division of the primary cell; the first primary derivative becomes the functioning antheridial cell. Again, if the first cell cut off contains sufficient protoplasm it may divide once or even twice to form as many as four free nuclei. When this occurs, the primary cell soon ceases to divide and begins to disintegrate (figs. N–Q and 28, 29, 33). The first primary derivative may function as an antheridial cell by directly dividing to form a spermatogenous and a sterile cell, by repeatedly dividing to form a number of free nuclei, or by becoming primary in nature and hence developing along with its sister cell to form a bi-antheridial gametophyte.

When the first primary cell is evanescent, a second primary division takes place. Nor is it uniform. Frequently the primary cell approaches the first primary wall before dividing, and it may come into contact with this wall. The second primary derivative is then cut off as a lenticular cell against the wall of the first and soon disintegrates (figs. C–E and 23, 25, 26). If, however, it remains imbedded in the cytoplasm of the primary cell, it divides to form a spermatogenous and a sterile cell (figs. F–I and 25, 38). Hence the second primary derivative may function as an antheridial cell.

When, as it has been hitherto described, the first and second primary derivatives are evanescent, a third primary division takes place, and the last cell cut off functions as the antheridial cell (figs. A–E and 42–51).

Since the spermatogenous cell may originate from the first, second, or third primary derivatives, we are forced to the conclusion that these cells are all potentially antheridial, one or in some cases two functioning as such. They may be known as evanescent or functioning antheridial cells, as the case may be.

Development; time; nutrition

Growth is exceedingly rapid; in three days the diameter of the pollen grain is doubled, its volume becoming four times as great. In *Pinus* "the mature pollen grain has the same size and form as

the microspore just prior to germination" (15). When two evanescent cells are cut off, these divisions take place before the increase in size. They follow one another in rapid succession; all stages of the first two primary divisions are to be found in the same sporangium. A resting period, that is, a period during which mitosis ceases, but during which there is a great increase in size and apparently in food supply, precedes the formation of the functioning antheridial cell, whether it be the first, second, or third primary derivative (figs. D, H, L, O, S and 30, 38, 44, 45). Since this last primary division and the secondary division to form the spermatogenous cell and the sister sterile cell are to be found in the same sporangium, it is evident that they are closely consecutive. The complete development is extremely rapid; on May 3 only one-celled stages were to be found, while on May 6, or sooner, the pollination stage had been reached. Trees on a sunny hillside shed the pollen at once; others retained it for ten days without further development. The functioning antheridial cell is imbedded in the cytoplasm of the primary cell, as shown above, and an increase in the size of the pollen grain precedes mitosis of the former cell. Evidently nutrition is a factor in determining the fate of an antheridial cell; in other words, whether the first, second, or third shall function as such.

Degeneration

In lenticular cells which contain a minimum of cytoplasm surrounding the nucleus, the latter does not pass out of telophase (fig. 26); the chromosomes contract, become globular, and finally disintegrate as irregularly granular masses (figs. 20, 21, 43), or accumulate at the periphery of the nucleus, giving it a vaginated appearance (fig. 45). When these cells collapse, double darkly-stained bands appear in cross-section. In *Picea canadensis* the intine does not imbed these degenerating cells. The first primary wall elongates as the pollen grain increases in diameter (figs. 37, 38, 50, 51); often it has the appearance of a third wall (fig. 50) which is attached to the intine near the origin of the wings. The disintegrating cell contents remain within the original walls; the latter meanwhile become elongated and thickened.

Degeneration may occur in any part of the gametophyte. Frequently the second primary derivative degenerates before the first (fig. 37). If sister primary cells are formed, the struggle resulting from their parallel development is generally so great that disorganization of both results (fig. 32). Usually one gains the ascendency. Often, after as many as three cells have been formed, one of the antheridial groups is crowded against the wall; irregular cavities appear in the cytoplasm (fig. 31); the protoplasm contracts and accumulates in masses of globules; and the nuclei becomes massed or uniformly granular (figs. 33, 53). When an extreme development of the first antheridial cell occurs, the primary cell as well as the secondary antheridial cell may disintegrate (fig. 33).

Mitoses

There are two types of mitoses; that characteristic of primary divisions, and that of secondary divisions. The latter does not differ essentially from ordinary somatic mitoses; the former is quite distinctive in its characters.

The nuclei and chromosomes are decidedly kinetic. Just before mitosis, the primary nucleus moves to a more or less polar position. Frequently it comes in contact with one of the primary walls (figs. 14, 15, 16, 43). There is the usual movement of the chromosomes to form the central plate at metaphase, and the separation of chromosome groups during anaphase. After the two nuclei have been formed, the one which is polar retains its position, while the primary nucleus moves to its central or supracentral position (figs. 5–7; 19–25; 43–45).

The changes in the volume of the nuclear space are very marked. During prophase a slight expansion is followed by a contraction (figs. 14, 15, 16, 28) which continues until the disappearance of the nuclear membrane. The chromosomes at the poles during early telophase aggregate into compact masses (figs. 4, 18, 47, 49); the nuclear membrane is formed, and the nucleus expands until it becomes three or four times its original size. There is an associated accumulation or disappearance of food particles in the surrounding protoplasm. This may be regarded as evidence in favor of LAWSON'S (7) explanation of similar phenomena, namely, that they are due

to osmosis. Moreover, the increase in size is most rapid when the nucleus is surrounded by most cytoplasm; the primary nucleus soon regains the size characteristic of the resting stage (figs. 6, 9, 18, 20, 21).

Chromatic structures

The resting nucleus contains several nucleoli. In early prophase "condensing bands" and "zig-zag threads" of chromatin become differentiated; definite looped chromosomes are formed (figs. 15, 35). Only in rare cases could their double nature be seen at this stage; it would seem that the halves remain rather closely appressed until metaphase. The nucleoli are present until late prophase (figs. 16, 35, 48). The chromosomes contract before aggregating in a definite cell plate (figs. 16, 48); during anaphase they are characteristically V-shaped (figs. 2, 3, 17). In early telophase compact chromatic aggregations are formed (figs. 4, 18, 41, 47, 49). These soon become irregularly vacuolate, and as the vacuoles increase in size, anastomosed bands of chromatin become differentiated (figs. 5, 22, 24, 43). The process is similar to that described by Sharp (8) in *Vicia*. The bands become more irregular in outline, and a number of nucleoli appear (figs. 23, 25, 44). As the number of nucleoli decreases, they become individually larger (figs. 20, 45, 6, 8, 46, 48, 50). The irregular bands are replaced by zig-zag threads and the nucleus passes again into the resting condition.

Achromatic structures

The achromatic structures in all primary divisions are most characteristic. The spindle fibers are inconspicuous during anaphase (figs. 2, 3, 36, 42); in many cases they can be distinguished only with difficulty (fig. 17) and are only slightly more definite than the vague radiations in the polar cytoplasm (figs. 3, 36). In early telophase there are very definite strands between the daughter nuclei. These are arranged in the form of a hollow cylinder (figs. 4, 18) which gradually broadens (figs. 19, 43) and moves toward the pole, partly enclosing the antheridial nucleus (figs. 5, 21, 24). Usually the fibers come in contact with the cell wall, the free ends swing outward, and so remain as curved or radiating strands

(figs. 7, 23, 44, 45). The cell plate forms late; it is most definite after the fibers have taken their final position (figs. 6, 7, 8, 41, 45). These mitoses are similar to that described for *Abies balsamea* (9).

The cell plate is evidently associated with the formation of the cell wall. In fig. 45 the arched fibers remain only at one side, but here there is a distinct cell plate and the cell wall is curved outward to meet it. When the wall is formed the fibers disappear. In many cases no cell plate could be seen (figs. 5, 21, 22, 23), and the division in the cytoplasm is continuous with the end of the fibers (fig. 25).

There is abundant evidence that the achromatic fibers are definite structures which change their position. The fact that there are groups of spindle fibers which have no immediate connection with nuclear membranes or chromosomes is further evidence for their individuality (fig. 8).

Secondary divisions have markedly different characters. A polar cap is formed during prophase (fig. 48); the spindle fibers are more strongly developed during metaphase (fig. 38); the cell plate forms early (figs. 47, 49), and the spindle fibers retain their original positions. The similarity of secondary divisions in the first and third primary derivatives is illustrated by figs. 47 and 49.

Chromatic extrusions

In the early stages of the gametophyte, darkly staining bodies occur in the cytoplasm. When the primary nucleus is in the resting stage, these bodies appear as spherical masses surrounded by a clearer area (fig. 34); when the primary cell is in active mitosis these extrusions become fragmented (fig. 35). These bodies originate from wandering chromosomes which escape during mitosis. Separate chromosomes are found near the primary cell wall during metaphase (fig. 36). Evidently these never take part in cell plate formation. They contract into spherical masses and wander into the cytoplasm. In other cases, during late anaphase, several chromosomes prematurely contract to form a more or less compact mass, thereby separating themselves from the chromosomes which later undergo a similar change (fig. 3).

Discussion

1. *Double pollen grains*

There are accounts of double pollen grains occuring in a number of species. Probably first was CHAMBERLAIN'S (10) description of *Lilium tigrinum*. "In about 20 cases there was a distinct wall dividing the microspore into two nearly equal parts." Both cells contained starch. His fig. 20 shows one of the cells containing two nuclei "which seem to represent generative and tube nuclei." One of the cells was regarded as prothallial, the other as antheridial. SCHAFFNER (11) found compound grains where two or more of the spores of a tetrad clung together (*Typha latifolia*). GUIGNARD (12) and Miss PACE (13) figure four microspores of an orchid within a common wall dividing to form tube nuclei and generative cells. COKER (13a) describes double grains in *Larix europea*. His fig. 6 is similar to my fig. 13; his fig. 8 corresponds to my fig. 12. He suggests that "the mother cell had only divided once, so that only two instead of four pollen grains were formed." In some of these grains "division proceeded as usual except that only one prothallial cell is evident" (cf. fig. 32). POLLOCK (3) has described a number of variations in the pollen grain of *Picea excelsa*. "In the material examined, the proportion of double pollen grains was found to be 2.4 per cent in a count of 1120. The three or four cells lying along the dorsal side of the pollen grain of this type do not constitute a prothallium or gametophyte of unusual size. They constitute the smaller portion of a pollen grain separated by a division wall into two nearly equal portions, each of which may form a typical antheridium." Double pollen grains have been variously interpreted. In *Picea canadensis* a study of the stages of development has shown that the two cells from which the double grain arises are the result of a primary division of the microspore (figs. 11, 13), and that one of these cells corresponds in origin to the more usual evanescent cell. All gradations between an equal division and one which cuts off a lenticular evanescent cell have been found (figs. 6, 7, 10, 11, 13). In the double pollen grain of *Picea* one of the antheridial groups is homologous with the usual evanescent polar cell.

2. "*Prothallial cells*"

Several species have been described in which the "prothallial" cell has the power of division. Among the number are *Ginkgo biloba*, by STRASBURGER (1); *Picea excelsa*, by MIYAKE (2) and POLLOCK (3); *Abies balsamea* (9); *Agathis*, by JEFFREY and CHRYSLER (14); *Podocarpus*, by COKER (13a); and *Dacrydium* by Miss YOUNG (16). The similarity of the generative cell and the prothallial cells is pointed out by Miss YOUNG: "In *Dacrydium*, as in *Podocarpus* and *Abietineae*, a third cell is cut off from the main body of the spore. It overlies the others and is so similar to them that, but for its subsequent behavior, one might think it a prothallial cell. It is the generative cell, generative in the sense that it is the ancestor of the spermatogenous cell. This and the second prothallial cell now divide." The first prothallial cell may also divide. Again: "at shedding the pollen grain contains the body cell and five free nuclei. The nucleus of the body cell is indistinguishable from those of the prothallial cells and the tube nucleus." POLLOCK (3) states that in a large proportion of the gametophytes of *Picea excelsa* there is only one prothallial cell. BURLINGAME (20) says: "in *Podocarpus* one primary prothallial cell may be cut off, after which the free nucleus divides to form the free spermatogenous cell and the tube nucleus; or two primary cells may be cut off before the tube nucleus is separated from the primary spermatogenous cell." In these two species, as well as in *Picea canadensis*, the antheridial function is not limited to a definite primary derivative. It has been established that "prothallial cells" and generative cells may be similar in appearance; that frequently they are similar in their power of division; the similarity is further emphasized by the presence of "prothallial cells" in the pollen tube. The present account has emphasized the similarity in the origin, and has shown that potentially there is a similarity in function; that any one or sometimes two of the primary derivatives may be antheridial. To what extent we are justified in suggesting that these phenomena are indicative of a multi-antheridial ancestral form only further research can determine.

Relationships

No one character is sufficient to establish relationships of plant groups. Since similarity of male gametophytes gives only one-sided evidence for the relationship of species or genera, the discussion will be limited to comparison of types. In Taxodineae and Cupressineae the gametophyte consists of an antheridial cell, which may divide before or after shedding, and a tube nucleus; no evanescent cell is present. Similar gametophytes are found in *Picea canadensis* (fig. 30). In cycads two cells are cut off from the primary cell, one of which is antheridial (cf. fig. 38). The shedding stage characteristic of the abietinean gametophyte (also of *Ginkgo* and *Ephedra*) contains two more or less evanescent cells and an antheridial cell, which may or may not divide, beside the tube nucleus (cf. figs. 50, 51, 52). The podocarp type is similar, but the polar cells are not evanescent and frequently divide (cf. figs. 39, 40, 52). A massive polar tissue containing free nuclei, which LOPRIORE (18) regards as antheridial, is characteristic of the araucarian type. A similar gametophyte is shown in fig. 33. The male gametophyte of *Picea canadensis* is in a state of unstable equilibrium. Slight differences in conditions are sufficient to shift the balance in one of several possible directions. The resulting forms correspond to the various types of gametophytes found in gymnosperms.

Summary

In the male gametophyte of *Picea canadensis*, one, two, or three potentially antheridial cells are cut off from the primary cell; one of these divides to form a spermatogenous and a sterile cell; the others, when formed, are more or less evanescent. Occasionally there are two functioning antheridial cells, resulting in a bi-antheridial gametophyte.

The writer is indebted to Professor JOHN M. COULTER, Dr. CHARLES J. CHAMBERLAIN, and Dr. W. J. G. LAND for many suggestions and criticisms.

UNIVERSITY OF CHICAGO

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EXPLANATION OF PLATES XV–XIX

The drawings (figs. 1–53) have been made with the aid of the Abbé camera lucida. The original magnification was 2000. A reduction of one-half has been made in reproduction.

FIGS. 1–13.—The first primary division; the primary cell retains its identity; the first primary wall cuts off an antheridial cell.

FIG. 1.—Primary cell.

FIG. 2.—A division at right angles to the longitudinal axis.

FIG. 3.—A division in the plane of the axis; a chromatic extrusion is being formed and is separating from the nuclear chromosomes.

FIGS. 4, 5.—Telophases: the primary nucleus and spindle fibers separating.

FIGS. 6, 7, 8, 11.—Telophases: each shows the cell plate in one of 4 positions; in fig. 8 both nuclei have escaped from the spindle fibers.

FIGS. 9, 12.—Two free nuclei in common cytoplasm; compare fig. 8.

FIGS. 10, 13.—The primary cell has given rise to two daughter cells, each of which may function as a sister primary cell.

FIGS. 14–27.—The second primary division.

FIG. 14.—The primary cell in contact with the first evanescent antheridial.

FIGS. 15, 16.—Prophases.

FIG. 17.—Anaphase: spindle fibers indistinct.

FIGS. 18–25.—Telophases: illustrate migration of primary nucleus and spindle fibers; fig. 24, a view from upper pole; figs. 23–25, the formation of the cell wall.

FIGS. 26, 27.—Pollen grains with primary cell and two non-functioning antheridial cells; compare size with fig. 1.

FIG. 28.—First antheridial ("prothallial") cell in division.

FIG. 29.—Primary cell; also first and second antheridial cells; the former has divided to form two nuclei.

FIG. 30.—First antheridial cell has divided to form a spermatogenous and sterile cell.

FIGS. 31, 32.—Two sister primary cells (cf. figs. 11 and 13) have given rise to a bi-antheridial gametophyte (disintegration).

FIG. 33.—Four free nuclei, products of first antheridial cell; the primary cell and second antheridial cell disintegrating.

FIGS. 34–36.—Chromatic extrusions.

FIG. 37—The position of the first primary wall and the large polar cavity is to be noted.

FIG. 38.—The second antheridial cell dividing.

FIGS. 39–40.—Laterally placed derivatives of first and second antheridial cells.

FIGS. 41–46.—The third primary division.

FIG. 41.—A polar view.

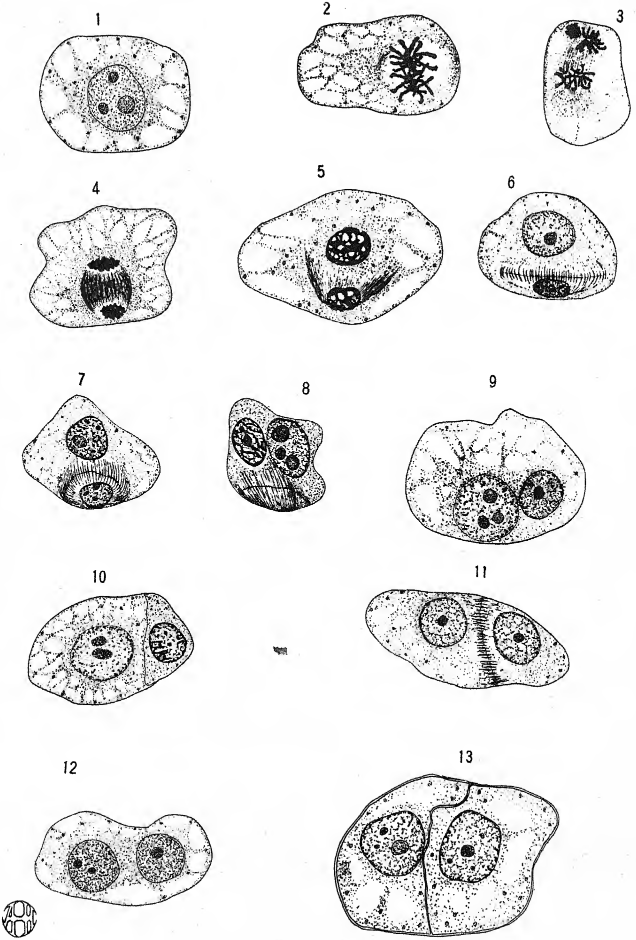
FIG. 46.—Large primary nucleus (tube nucleus): the third antheridial cell just before mitosis; the second in normal condition except for compression; the first disintegrated.

FIG. 49.—Mitosis in the first antheridial cell (cf. fig. 28).

FIGS. 47, 48, 50, 51.—Division of the third antheridial cell to form a spermatogenous and a sterile cell.

FIG. 52.—Showing polar cells, the products of secondary divisions.

FIG. 53.—Shows disintegrating derivatives of the first antheridial cell; a sterile cell; a spermatogenous cell and the primary nucleus (or tube nucleus).



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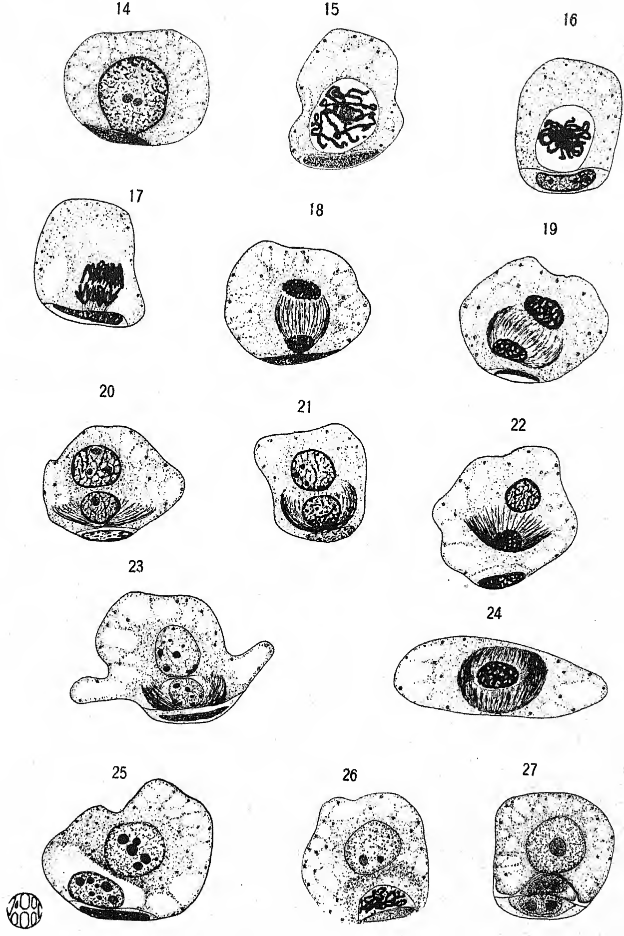

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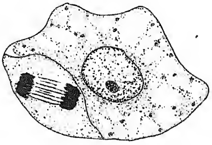

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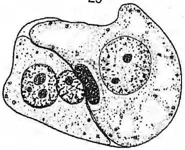

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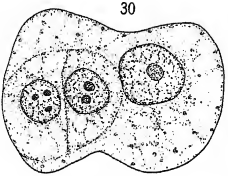

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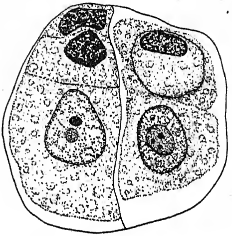

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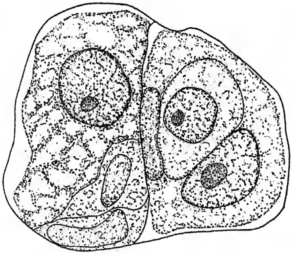

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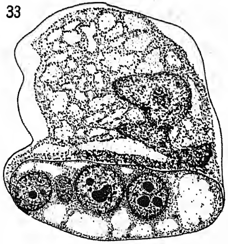

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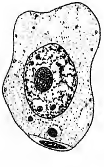

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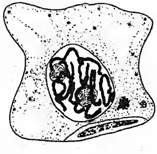

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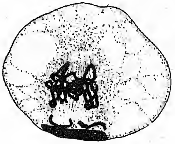

HUTCHINSON on PICEA

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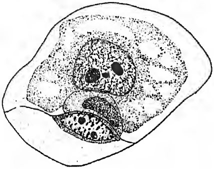

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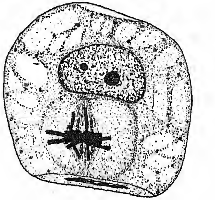

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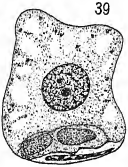

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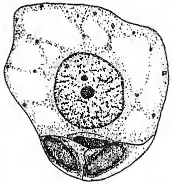

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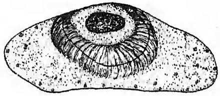

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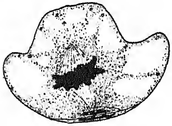

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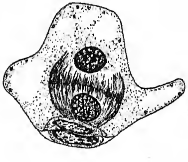

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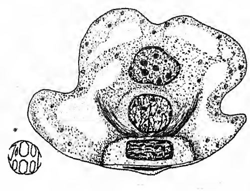

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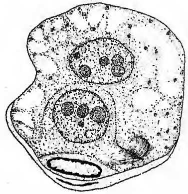

HUTCHINSON on PICEA

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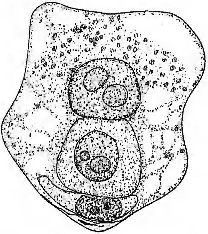

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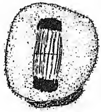

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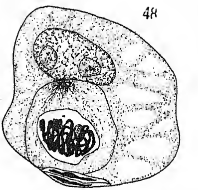

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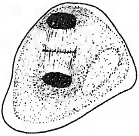

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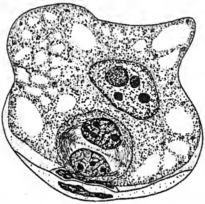

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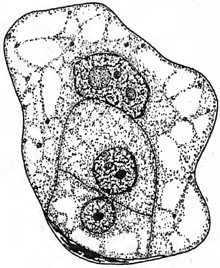

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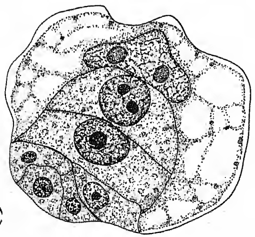

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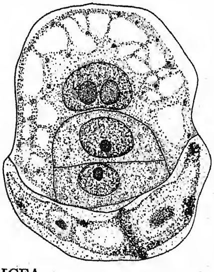

HUTCHINSON on PICEA

STUDIES IN THE GENUS BIDENS. II

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 201

EARL E. SHERFF

(WITH THREE FIGURES)

Bidens acuticaulis, sp. nov.—Herba annua, tenerrima, 4–8 dm. alta; caule acute angulato, ramoso; ramis adscendentibus, acute angulatis et fere subalata, remote pubescentibus. Folia opposita, petiolata, petiolo adjecto 1–4 cm. longa, pinnata aut irregulariter bipinnata; foliolis (et lobis) linearibus, 0.5–1 mm. latis. Petioli 2–10 mm. longi. Capitula multa, terminalia, 3–4 mm. alta et 2.5–3.5 mm. lata (frutescentia demum 1–1.2 cm. alta et 4–5 mm. lata), ligulata. Involucrum basi pubescens; squamis duplici serie dispositis; exterioribus linearibus, plus minusve pubescentibus, 3–4 mm. longis; interioribus lanceolatis, marginibus diaphanis 1.5–3 mm. longis. Ligulae circiter 4, parvae, sub-flavidae aut fere albae, 2– (4–) striatae, 3–5 mm. longae. Paleae lineare-lanceolatae, striatae, marginibus diaphanis. Achaenia linearia, remotissime pubescentia, margine adscendente-ciliato, apice erecto-hispido et biaristato aristis retrorsum hamosis, 6–12 mm. longa.

John Gossweiler 4052, in herb-grown woods, Angola, April 4, 1906 (type in Herb. Brit. Mus.).

Bidens rufovenosa, sp. nov.—Herba erecta, perennis (?), 4–6 dm. alta; caule subtereto, striato, glabro, plus minusve ramoso; ramis (aut ramulis) monocephalicis. Folia opposita, petiolata, petiolo adjecto 4–6 cm. longa, pinnata, spinuloso-ciliata, supra sparsim et brevissime spinuloso-pubescentia, infra sparsim pubescentia et ad venas minute rufo-tomentosa;[1] foliolis lateralibus lanceolatis (aut foliorum superorum lineare-lanceolatis), incisis aut longe dentatis; foliis supremis integris et lanceolatis, aut ternatis, foliolis integris et lanceolatis; petiolis basi connatis, foliorum

[1] Dr. S. ECKERSON, of the University of Chicago, has kindly made for me a critical examination of a leaf from the type, and she reports the color of the pubescence due to a red stain produced in the mucilage cells along the veins.

inferiorum angustis et 1–1.5 cm. longis, foliorum superorum brevioribus aut absentibus. Capitula ligulata, frutescentia 1–1.3 cm. lata et 0.8–1 cm. alta. Involucri squamae membranaceae, margine diaphanae, duplici serie dispositae; exterioribus (7–8) linearibus, 3–5 mm. longis; interioribus lanceolatis, dimidio longioribus. Ligulae (in specim. exsicc.) aurantiacae, anguste lanceolatae, ápice integrae, ad basem minute hispidae, 7–9-lineatae, 1.5–1.8 cm. longae. Paleae membranaceae, lineares, margine diaphanae, 6–8 mm. longae. Achaenia linearia, subplana, una facie valde unicostata, adscendente-ciliatae, supra adscendente-hispidae, biaristatae aristis brevibus et nudis, 8–9 mm. longa.

John Gossweiler 4176, among the ferruginous rocks near the fort at Kabango, Princip. Amelia, Africa, Dec. 1907 (type in Herb. Brit. Mus.).

Bidens cinerea, sp. nov.—Herba, annua (?), erecta aut adscendente-erecta, cinerea; caule striato, squarroso-hispido, subtetragono, monocephalico (aut ramis monocephalicis). Folia opposita, petiolata, tuberculato-scabrida et minute spinuloso-setosa, ternata aut pinnata, petiolo adjecto 2–6 cm. longa; lobis dentatis aut lobulatis, ovatis aut lanceolatis, brevioribus paulo aut etiam dimidio quam petiolis; petiolis spinuloso-setosis, 0.5–2 cm. longis. Capitula terminalia, ligulata, pedunculis 6–10 cm. longis, flosculis hermaphroditis exsertis. Involucrum hispidum, squamis duplici serie dispositis; exterioribus circa 8, linearibus, 4–5 mm. longis; interioribus dimidio longioribus, lanceolatis, margine diaphanis. Ligulae flavae, lineari-lanceolatae, apici integro, 7–9-striatae, circa 1.2 cm. longae. Paleae lineares, membranaceae, margine diaphanae, circa 8 mm. longae. Achaenia linearia, nigra, compressa, uno facie unicostato, striata, adscendente-hispida, biaristata, aristis retrorsum hamosis, circa 1.1 cm. longa.

Lieut. *C. S. Smith*, May, 1893, Kilimanjaro, Africa (type in Herb. Kew).

Bidens punctata, sp. nov.—Herba annua, erecta, gracilis, ±5 dm. alta, caule subtetragono, glabro (aut nodis sparsissime longo-piloso), striato, supra plus minusve sparsim ramoso; ramis subtetragonis aut teretis, tenuibus, striatis, glabris (nisi nodis pilosis); ramulis monocephalicis. Folia opposita, tripartita (infima et suprema integra), reflexa luce nigro-punctata demum, ciliata, supra sub-

glabra, infra pallidioria et sparse longo-hispida; lobis lateralibus brevibus, linearibus aut lanceolatis, integris (aut non saepe ad basim lobatis), basi saepe subangustatis; lobo terminali elongato, lineari aut lineari-lanceolato, integro (aut ad basim 1–2-dentato); petiolis plus minusve hispidis, basi connatis, 0.4–1 cm. longis. Capitula terminalia, longe (6–12 cm.) et tenuiter pedunculata, ligulata, capitula ligulata 2 cm. lata. Involucrum basi hispidum, squamis duplici serie dispositis; exterioribus (5–8) lineari-spathulatis, glabris aut hispido-ciliatis (aut etiam sparse hispidis), 2–4 mm. longis; interioribus lanceolatis, membranaceis, striatis, margine diaphanis, sparsim hispidis, 5–8 mm. longis. Ligulae flavae, lineari-lanceolatae, apice integro, 5–9-lineatae, 8–10 mm. longae. Paleae lineares, striatae, membranaceae, margine diaphanae, demum 6–7 mm. longae. Achaenia linearia, compressa, nigra, marginibus et faciebus tuberculatis aut tuberculato-hispidis, apici hispido pectinatim, biaristata, aristis 1–2 retrorsum hamosis.

Archdeacon *W. P. Johnson* 343, Tumbi (Makapula), April 27 (1901?; type in Herb. Kew); *idem* 341, Tumbi hill, Tumbi, April 27 (1901?).

The description here given is drawn mainly from the type sheet in Kew Herbarium, although certain dimensions, etc., are taken from *Johnson* 341 (also at Kew), a sheet with a larger and more matured plant. It may be remarked here that the punctate character noted above for the leaves is to be met with in *Bidens Taylori* and *B. Baumii* (see below), also a small number of other species.

Bidens vincaefolia Karsten et Schultz Bipontinus, sp. nov.—Herba, perennis (?), volubilis (?), caule angulato aut tetragono, ramoso; ramis glabris aut subglabris, tetragonis, striatis. Folia opposita, petiolata, petiolo adjecto 2–3 cm. longa, tripartita aut raro indivisa, subcoriacea aut membranacea, subtus pallidiora et ad venas minute pubescentia, marginibus integris ("rarius serrata" ex Karst. et Schz. Bip.) et in specimine sicco subrevolutis; foliolo terminale lanceolato, 1–1.8 cm. longo; foliolis lateralibus oblanceolatis aut obovatis, 0.6–1.3 cm. longis; petiolo ciliato, 1–1.8 cm. long. Capitula pedunculata, ligulata, circiter 1 cm. lata. Involucrum basi hispidum; squamis duplici serie dispositis et subaequalibus; exterioribus circiter 5, linearibus, plus minusve pubescentibus, nigro-striatis, 3–4 mm. longis; interioribus

lanceolatis, subglabris. Ligulae circiter 6 aut 7, flavae, nigro-striatae nonnullis lineis, 5–8 mm. longae. Paleae lineares, striatae, marginibus diaphanis. Achaenia linearia, biaristata aristis retrorsum hamosis, circiter 1 cm. longa.

Karsten, Bogota, Columbia (type in Herb. Mus. Hist. Nat. Paris).

This species appears very unique, being distinct in general aspect from any other species known to me. The type sheet, originally in SCHULTZ BIPONTINUS' own herbarium, bears several small specimens. These are remarkably uniform and are allied most nearly with *Bidens rubifolia* H. B. K., the leaves of which are much less diminutive. The label bearing the specific name here set forth has also the date Dec. 18, 1856, which, from its position on the label, may be that of the determination rather than the collection. While true *Bidens rubifolia* grows at Bogota (e.g., *I. F. Holton* 365, Oct. 28, 1852), KARSTEN and SCHULTZ BIPONTINUS evidently regarded the Karsten plant specifically distinct. This course I also must adopt, as I am unable to connect the two forms in herbaria by any intermediate forms.

BIDENS ODORATA Cav. Icon. et Descript. Plant 1:9. *pl. 13*. 1791; *Coreopsis ferulaefolia* Jacq. var. *odoratissima* Pers. Synops. Plant. 2:477. 1807; *Bidens ferulaefolia* DC. var. *odoratissima* DC. Prodr. 5:603. 1836.—For many years, PERSOON'S variety *odoratissima* of *Coreopsis ferulaefolia* seems to have been unfamiliar to botanists. DECANDOLLE (*l. c.*) merely mentioned it: "quid sit var. *odoratissima* à Persoonio citata ignoro." PERSOON'S variety was founded on CAVANILLES' plant in JUSSIEU'S herbarium and was said to be native to Peru. This cited specimen I have not found, but there exists in the British Museum a good specimen from CAVANILLES, labeled "*Coreop[s]is odoratissima* nobis, Mexico," which matches PERSOON'S description precisely. The leaves, probably because of having grown under cultivation, are especially slender-divided and give a superficial resemblance to those of *Bidens ferulaefolia* (Jacq.) DC. The specimen is identical with two other specimens (in Herb. Brit. Mus.) from the herbarium of CAVANILLES, one labeled "*Coreopsis odorata* olim *Bidens*," and the other "*Bidens odorata* Cav. Ic. V. 1, nunc *Coreopsis*." Both of these last two are further labeled, in pencil, "*Cosmos odoratus*."

A study of other mature specimens of *Bidens odorata* shows that it is a Mexican species primarily (if not indeed exclusively), and is not at all a *Coreopsis*, but rather a true *Bidens*, although

having achenes sometimes approaching those of *Cosmos* and with rays varying, in herbarium specimens, from yellow to white, rose, or violet (a single sheet frequently showing all these variations, as, for example, *Palmer* 674, Herb. Kew).

During the past few years, certain writers, notably OLIVER and HIERN, S. L. MOORE, and O. HOFFMANN, have described a considerable number of striking and positively new species of *Coreopsis* and *Bidens* from various parts of Africa. In their generic distinctions, however, they have unfortunately been guided mainly by the direction of the barbs on the awns of the achenes, or, in certain cases, they have had access at the time to immature achenes only. Regarding the latter point, the disadvantage and source of error are at once evident on examining the real distinctions between *Coreopsis* and *Bidens*. As will be seen below, the presence of two more or less conspicuous lateral wings upon the achenes seems by far the most nearly constant character of *Coreopsis* as distinguished from *Bidens*. But in several species of *Coreopsis* these wings are completely lacking on the immature achenes. Thus, species described from immature specimens as *Bidens* may later prove to be *Coreopsis*. Again, a species erroneously described from immature specimens as *Coreopsis*, because of a recognized resemblance to some particular species of *Coreopsis* and disregarding the absence of wings, must thereafter be treated by botanists as *Coreopsis* until mature material can be obtained to prove its status as true *Bidens*.

Concerning the direction of the barbs on the awns of the achenes, however, a more detailed statement is needed. Until comparatively recent times, botanists referred to *Bidens* those species with retrorsely barbed awns, and to *Coreopsis* those species with antrorsely barbed awns or with awns inconspicuous. But, from time to time in North America, new forms have been discovered, identical in each case with a certain species of *Coreopsis* (as then delimited) or *Bidens* except in the direction of the barbs on the awns. ASA GRAY (cf. FERNALD, Rhodora 15:77. 1913), when confronted with such a form of "*Coreopsis aristosa* Michx." (the then accepted name), designated it "*C. aristosa* in Bidentem transformata." Later (Synop. Fl. N. Amer. 1^{II}:294–296. 1884) he treated this

and similar forms as hybrids between *Coreopsis* and *Bidens.* Still later, BRITTON (Bull. Torr. Bot. Club **20**:280–281. 1893), emphasizing the instability of the barb-direction character for *Bidens frondosa* L., and also separating the two genera on general grounds rather than by one particular character, transferred six species from *Coreopsis* to *Bidens.* The validity of these transfers has since been accepted unhesitatingly by all prominent American botanists who have critically studied the Eastern United States species of *Bidens,* among them WIEGAND (Bull. Torr. Bot. Club **26**:401. 1899), GREENE (e.g., Leafl. Bot. Crit. **1**:200.[2] 1906), and ROBINSON and FERNALD (GRAY'S Man. ed. 7. p. 839. 1908). It is also implied by many other American botanists working upon the species of other regions but following the same distinctions, a singular case being that of BRANDEGEE'S description (Zoe **5**:239. 1908) of *Bidens alpina* and GREENMAN'S description (Proc. Amer. Acad. **41**:264. 1905) of *Bidens sarmentosa.* The achenes are described for *B. alpina* as "nearly smooth; awns none or two varying from 1 mm. long to rudiments, corneous and not barbellate"; for *B. sarmentosa,* as "glabrous or sparingly hispidulous, awnless or with reduced awns." But, on investigation, these species are found to be identical; in turn, *B. sarmentosa,* which from priority of publication would otherwise stand as the accepted species, is found to match[3] in every character *Coreopsis anthemoides* DC., having achenes described (DC. Prodr. **5**:573. 1836) as "(immaturis) linearibus glabris brevissime bidentatis." Thus, a species placed by DECANDOLLE in *Coreopsis,* evidently because of its very short awns, was independently referred by BRANDEGEE and by GREENMAN, about 70 years later, to *Bidens,* evidently

[2] Thus, GREENE refers to *Bidens* a plant ("*B. tenuissima*") with "erect, *upwardly barbed awns.*"

[3] I have examined several authentic cotypes of *B. alpina,* the type and numerous authentic cotypes of *B. sarmentosa,* also several cotypes of "*Coreopsis anthemoides*" (*Bidens anthemoides* Sherff, BOT. GAZ. **56**:493. 1913). War conditions in Europe have precluded for the present my examination of DECANDOLLE'S type at Geneva. However, the characters cited by DECANDOLLE and his comparison of this species with *Anthemis arvensis,* which it at times simulates in habit to a remarkable degree, leave no doubt that the cotypes examined (in Herb. Brit. Mus. and elsewhere) truthfully represent the type.

because the achenes lack wings and the general characters coincide closely with those of certain unquestioned species of *Bidens* (e.g., *B. humilis* H. B. K., with awns retrorsely barbed).

Fernald, in a recent discussion of the awn characters of *Bidens* (Rhodora 15: 74–78. 1913), lists no less than six American species in which occurs a more or less pronounced form having the awns barbed in the reverse from the normal direction. Thus, for example, *Bidens connata* Muhl. has awns retrorsely barbed, while var. *anomala* Farwell has awns antrorsely barbed. Again, *Bidens aristosa* (Michx.) Britton has antrorsely barbed awns, while the probably valid var. *Fritcheyi* Fernald has retrorsely barbed awns. Hence it is obvious that, were the old artificial method of distinguishing between *Coreopsis* and *Bidens* (namely by the direction of the barbs on the awns) to be retained, an anomalous situation would result. We should be compelled either to regard each of these varieties as a hybrid between two species of distinct genera, a course certainly unwarranted in several cases (cf. Fernald, *l. c.*, and Wiegand, Bull. Torr. Bot. Club **26**: 401. 1899), or to refer each variety to the other genus, an entirely indefensible alternative. We are compelled, then, to view these varieties, in at least the majority of cases, as merely more or less distinct and pronounced forms of their respective species. This being true, the awn character method of separating *Coreopsis* from *Bidens* proves utterly worthless, and must be permanently abandoned, as it indeed has been by American botanists.

On reference to recent descriptions and types of African species of *Bidens*, we find that in several cases the awns, even on achenes on the same head, are barbed both antrorsely and retrorsely. Thus, for example, Moore (Jour. Linn. Soc. Bot. **37**: 322. 1906) created the name *Bidens ambigua* for *Gossweiler* 1189, precisely for the reason that some of the awns are smooth, others antrorsely barbed, and others retrorsely barbed ("achaeniis . . . . aristis 2 quam se ipsa brevioribus dentibus perpaucis nunc erectis nunc recurvis onustis vel etiam omnino calvis . . . . , hence the trivial name"). Yet in the same year (Jour. Bot. **44**: 22. 1906) he likewise somewhat arbitrarily created the name *Coreopsis Taylori* for a plant showing the same variation (coll. *W. E. Taylor*, Jan. 5, 1886;

"achaeniis . . . apice setuloso-ciliatis calvis vel aristulis 1 vel 2 brevissimis erecto—vel patenti—vel etiam recurvo—uncinulatis onustis saepe vero nudis . . . ; indeed, the plant might almost as well be considered a *Bidens*, but the habit is that of *Coreopsis*"). In referring the latter species to *Coreopsis*, he relied mainly upon its habital similarity to other (so-called) species of *Coreopsis* from Africa. But, as will be seen presently, some of these species belong in reality to *Bidens*. Therefore, this habital similarity, affording formerly an apparently good reason for the name *Coreopsis Taylori*, can no longer be given much consideration.

The present writer, in bringing together the numerous species of *Bidens* for monographic treatment, has come to adopt fully the generic limits of these two genera as followed by recent American botanists. In brief, the genus *Coreopsis* is maintained primarily because of the peculiar habit and winged achenes of the Linnaean type species (excluding *C. alba*, *C. Bidens*, and *C. alternifolia*, Sp. Plant ed. 1. pp. 907–909. 1753; cf. BRITTON, Bull. Torr. Bot. Club **20**:280. 1893). Similarly, the genus *Bidens* is maintained primarily because of the peculiar habit, strongly barbed awns, and wingless achenes of several of the Linnaean type species of *Bidens*. Among the species of either small group a fair degree of uniformity in several characters occurs. But on extending our observations to other species of *Coreopsis* and *Bidens*, we find remaining no absolute uniformity in even one distinctive character. However, one such character does persist to a surprising extent. It is the presence (in *Coreopsis*) or absence (in *Bidens*) of two lateral wings upon the mature achene. Among so many species from widely remote regions does this character separate two genera with different aspects that, *in cases where other criteria are absent*, it appears to offer the only logical basis of distinction. Accordingly, and with a view to thus delimiting these two genera more accurately, notably among the African species (where the generic limits tend to overlap) this basis of distinction is here adopted. As a consequence, it is found necessary to make the following transfers for the flora of Africa. This list includes only those species upon the types or other positively authentic material of which I have personally examined the mature achenes.

Bidens arenicola (S. Moore), comb. nov.—*Coreopsis arenicola* S. Moore, Jour. Linn. Soc. **37**: 170. 1905.

Bidens Grantii (Oliver), comb. nov.—*Coreopsis Grantii* Oliver, Trans. Linn. Soc. **29**: 98. *pl. 65*. 1873.

Bidens grandis, nom. nov.—*Coreopsis speciosa* Hiern, Cat. Welw. Afr. Pl. **3**: 585. 1898.

Bidens Kirkii (Oliver and Hiern), comb. nov.—*Coreopsis Kirkii* Oliver and Hiern, Fl. Afr. Trop. **3**: 390. 1877.

The type material (in Herb. Kew) lacks mature achenes, but the mature material of *G. F. Scott Elliot* 6909, referable to this species, has the achenes of a true *Bidens*.

Bidens ambacensis (Hiern), comb. nov.—*Coreopsis ambacensis* Hiern, Cat. Welw. Afr. Pl. **3**: 586. 1898.

Bidens ugandensis (S. Moore), comb. nov.—*Coreopsis ugandensis* S. Moore, Jour. Linn. Soc. **35**: 347. 1902.

Bidens ruwenzoriensis (S. Moore), comb. nov.—*Coreopsis ruwenzoriensis* S. Moore, Jour. Linn. Soc. **35**: 345. 1902.

Bidens kilimandscharica (O. Hoffm.), comb. nov.—*Coreopsis kilmandscharica* O. Hoffm., Bot. Jahrb. **20**: 234. 1894.

Bidens Schweinfurthii, nom. nov.—*Coreopsis linearifolia* Oliver and Hiern, Fl. Afr. Trop. **3**: 390. 1877.

This species is here given a new name to avoid any possible confusion with *Bidens linearifolia* Schz. Bip., which, in fact, is a true *Cosmos* and has been renamed correctly *C. linearifolius* Hemsley (Biol. Centr. Amer. **2**: 200. 1881).

Bidens Taylori (S. Moore), comb. nov.—*Coreopsis Taylori* S. Moore, Jour. Bot. **44**: 22. 1906.

Bidens insecta (S. Moore), comb. nov.—*Coreopsis insecta* S. Moore, Jour. Bot. **46**: 42. 1908.

Bidens Baumii (O. Hoffm.), comb. nov.—*Coreopsis Baumii* O. Hoffm. in H. Baum Kun.-Sambes. Exped. 419. 1903.

Bidens Elliotii (S. Moore), comb. nov.—*Coreopsis Elliotii* S. Moore, Jour. Linn. Soc. **35**: 346. 1902.

BIDENS FLORIBUNDA H. B. K., Nov. Gen. **4**: 238. 1820; *Bidens simplicifolia* Wright, Kew Bull. 1906: 5. 1906.—The type of *B. simiplicifolia* Wright (in Herb. Kew) is identical with the type

specimen of *B. floribunda* H. B. K. (in Herb. Mus. Hist. Nat. Paris).[4] An important feature of *B. floribunda* is its simple leaves, although KUNTH, in the original description, betrayed doubt as to whether tripartite leaves are completely lacking (foliis . . . simplicibus, nisi folia inferiora, a me haud visa, in hac quoque specie ternata sint"). WRIGHT, in describing *B. simplicifolia* ("a speciebus reliquis Austro-Americanis foliis indivisis ovatis acuminatis differt"), was clearly unaware that this same species had already long before been described from the same country (Ecuador) as *B. floribunda*. An excellent cotype of *B. simplicifolia* in the Herbarium of Field Museum has foliage much superior to that of the type. It possesses four pairs of large simple leaves, the lowermost ones 15.2 cm. long and 5.6 cm. wide. From these it would appear even more plausible that the species is constantly simple leaved.

BIDENS ALAUSENSIS H. B. K., Nov. Gen. 4:184. 1820; *Bidens valparadisiaca* Colla, Mem. Accad. Torin. **38**:12. *pl. 24*. 1835; *Bidens chilensis* DC., Prodr. **5**:603. 1836.—A study of several authentic specimens of *B. chilensis* DC. collected around Quillota, Chile, by BERTERO about 1829, shows that these are precisely the same as the type specimen (fig. 1) and BONPLAND'S private duplicate specimen of *B. alausensis* H. B. K. (both specimens in Herb. Mus. Hist. Nat. Paris). Other specimens, collected at various dates by GAY, W. H. HARVEY, BRIDGES, etc., and all determined as *B. chilensis*, show that the rays are frequently white, as stated by BERTERO (DC., *l. c.*), instead of yellow, as in DECANDOLLE'S type specimen.

Regarding *B. valparadisiaca* Colla we need only to say that it was founded upon BERTERO'S material, as was DECANDOLLES *B.*

[4] The only difference that I can detect is that, in at least its cotype material examined, *B. simplicifolia* has the exterior involucral bracts mainly subspatulate and only slightly ciliate; in the type of *B. floribunda*, these are more oblong and more ciliate. While such a variation in shape or size of these bracts has already, in some species, been made the basis for a varietal distinction (e.g., *B. rosea aequisquama* Fernald, Proc. Amer. Acad. **43**:68. 1907), such a course would seem undesirable here. Aside from the fact that these bract characters frequently vary, in other better known species of *Bidens*, from oblong to spatulate on the same head, it would mean the unwelcome use, according to rules, of the name *simplicifolia* for a variety of a species that itself is simple leaved (*simplicifolia*).

chilensis,[5] and hence likewise must be synonomized with *B. alausensis*.

It is interesting to note that *Bidens crithmifolia* H. B. K. was suspected by SPRENGEL (Syst. Veg. ed. 16^{III}. p. 454. 1826) of belonging to *B. alausensis*. A study of the types of both species shows, however, that they are entirely distinct.

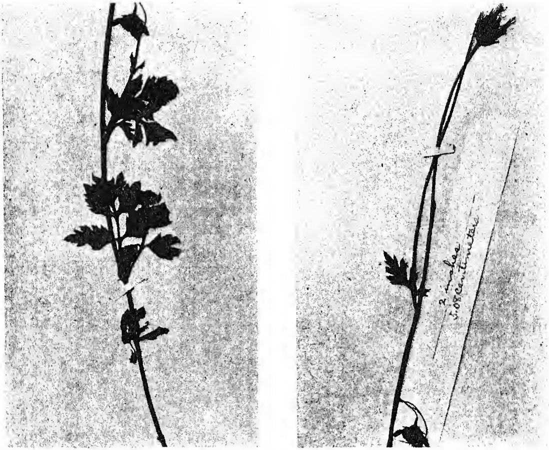

FIG. 1.—Type of *Bidens alausensis* H. B. K. in Herb. Mus. Hist. Nat. Paris; the two photographs slightly overlap.

BIDENS TENELLA L., Amoen. Acad. 6:96. 1763.—This species, was included by DECANDOLLE (Prodr. 5:605. 1836) among the "species non satis notae." A sheet with two specimens exists however, in the Linnaean Herbarium. These are in good condition and show at once that the species is in no way a *Bidens*, and

[5] *B. valparadisiaca* was based (COLLA, *l. c.*) upon a specimen from Valparaiso, while *B. chilensis*, which DECANDOLLE himself equated with it, was based upon a specimen collected in the immediate vicinity, but to the northeast ("circa Quillota," DC., *l. c.*).

may even be founded upon two distinct plants from widely separate parts of the world. In fact, the sheet bears a determination by SCHULTZ BIPONTINUS, naming the plant "ad sinistram *Pectis* (*tenella*)" and that "ad dextram Charieis *heterophylla* Cass."

BIDENS ANDONGENSIS Hiern, Cat. Welw. Afr. Pl. 1^{III}:588. 1898.—At the time of describing this species, HIERN confessed himself uncertain as to its generic status, owing to the insufficient material. Since then, however, an admirable, well developed specimen (*John Gossweiler* 3631, Angola, August 3, 1907) has been received at the Herbarium of the British Museum. This matches sufficiently in each detail the plant fragment and drawings on the type sheet, at the same herbarium, and proves conclusively that the species is a true *Bidens*.

Bidens elata, comb. nov.—*Bidens cernua* L. var. *elata* Torr. and Gr., Fl. N. Amer. **2**:352. 1842; *Bidens dentata* Wieg., Bull. Torr. Bot. Club **26**:412. 1899, non *Bidens quadriaristata* DC. var. *dentata* Nutt.; *Bidens amplissima* E. L. Greene, Pittonia **4**: 268. 1901. —An excellent specimen of this species, collected by SCOULER at the Straits of De Fuca, is in the Torrey Herbarium, now included in the Herbarium of the N.Y. Bot. Garden. It is identical with the Scouler specimen of HOOKER'S herbarium (now in Kew Herb.), a specimen referred by HOOKER (Hook. Fl. Bor. Amer. **1**:314. 1833) to *B. chrysanthemoides* Michx. (but entirely distinct from Michaux's two type specimens in Herb. Mus. Hist. Nat. Paris). It is identical also with the type and cotype specimens of *B. amplissima* Greene. However, it is very different from the type (fig. 2) of *B. quadriaristata* DC. var. *dentata* Nutt. (in Herb. Brit. Mus.), a plant cited synonomously by TORREY and GRAY, but probably never seen by them, as indeed their failure to use their customary exclamation marks would partly imply.

WIEGAND, following TORREY and GRAY'S treatment, likewise equated these two plants, but GREENE (*l. c.*), who, however, had not seen NUTTALL'S type, justly denied their identity. Still GREENE'S name *B. amplissima* is superfluous according to rule of nomenclature, and the name *elata*, supported by a description ("leaves . . . . unequally and incisely serrate," etc., Torr. and

Gr., *l. c.*) that very definitely characterizes the cited specimens,[6] must be used for this species.

Bidens aurea, comb. nov.—*Coreopsis aurea* Ait., Hort. Kew. **3**: 252. 1789; *Bidens coronata* auct., non L.—In a former article

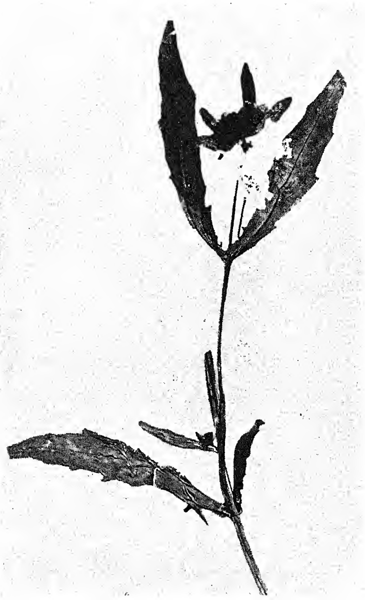

FIG. 2.—Type of *Bidens quadriaristata* DC. var. *dentata* Nutt., in Herb. Brit. Mus.

(BOT. GAZ. **56**:495. 1913), it was stated that BRITTON and not FISCHER was the one to transfer *Coreopsis coronata* L. to *Bidens*. An inspection, furthermore, of BRITTON's article cited (Bull. Torr.

[6] At least the Scouler specimens. I have not seen the Douglas specimens.

Bot. Club 20:280. 1893) shows that he was merely treating a group of *Coreopsis* species of GRAY'S *Synoptical flora* en masse, and was concerned with their generic rather than specific status. And study of GRAY'S descriptions and notes (GRAY, Synop. Fl. N. Amer. 1^{II}: 294. 1884) shows that GRAY equated, though somewhat provisionally, *Coreopsis aurea* Ait. with *C. coronata* of the Linnean Herbarium, a view retained as well by BRITTON (BRITTON and BROWN, Illustr. Fl. 3:498. 1913).

My search through the material of *Bidens* and nearly allied genera at Kew Herbarium failed to reveal an original specimen of *Coreopsis aurea* Ait., but a good and authentic sheet from Kew Gardens, in 1785, occurs in the Herbarium of the British Museum. It is the form recently treated by authors as *Bidens coronata* (e.g., BRITTON and BROWN, *l. c.*). In the Linnaean Herbarium, moreover, there still exists the original superb specimen of *Coreopsis coronata* L. It lacks mature achenes, but its several beautiful 8-rayed heads, with the rays strikingly well arranged on the paper (fig. 3), leave no doubt that LINNAEUS had this specimen at hand when describing *C. coronata* (Sp. Plant. ed. 2. 2:1281. 1763; "radio magno, octopetalo," etc.). While, indeed, LINNAEUS cited in his synonomy plants of VAILLANT and of PLUMMIER, these have been justly excluded by subsequent authors. Thus, for forming a true conception of *C. coronata* L., there are left for us the Linnaean specimen and description. The latter, by itself, is inadequate. The former, in GRAY'S time, seemed likewise disappointing, as being too nearly intermediate between *C. aurea* Ait. and *C. trichosperma* Michx. of his *Synoptical flora*. But, in later years, numerous specimens of these last two species have been added to our American herbaria and show very clearly differences in leaf outlines that GRAY, with his scantier material, could not properly define. A comparison with these specimens shows at once that the Linnaean type is the *Coreopsis trichosperma* Gray (*l. c.*), and hence *Bidens trichosperma* Britton.[7]

[7] Since the foregoing lines were written, Dr. N. L. BRITTON has kindly written me that many years ago he examined the Linnaean type, but, while entertaining doubts as to its true status, felt constrained, for want of achenes, to follow GRAY'S treatment, except as to generic affiliations. However, Dr. M. L. FERNALD has just informed me that GRAY'S fragment at Gray Herbarium, from the Linnaean Herbarium (where certain heads are missing on the single type specimen), "shows perfectly characteristic fruit of *B. trichosperma*, not of *B. coronata* of recent authors," thus confirming my conclusions in a most emphatic way.

My inability to find MICHAUX's specimen of his *Coreopsis trichosperma* in the Michaux collection at Paris (in Herb. Mus. Nat.

FIG. 3.—Type of *Bidens coronata* (L.) Britton in Herb. Linn.

Hist. Paris) makes it impossible to state definitely whether MICHAUX's specimen was *Bidens aurea* (Ait.), as here introduced, or

B. coronata (L.) Britton. Fortunately, lack of priority in either case precludes the use of MICHAUX's name *trichosperma*, and we thus are able to use names—*B. aurea* (Ait.) and *B. coronata* (L.) Britton—that are supported with authentic type or cotype specimens still extant and in a good state of preservation.

The plant with leaves divided into linear segments (*Diodonta leptophylla* Nutt., Trans. Amer. Phil. Soc. II. **7**:360. 1841; *Bidens coronata* var. *leptophylla* Mohr, Contrib. U.S. Nat. Herb. **6**:808. 1901) must properly be designated *Bidens aurea* (Ait.), var. **leptophylla** (Nutt.), comb. nov. NUTTALL's original specimen is still preserved in excellent condition (in Herb. Brit. Mus.).

UNIVERSITY OF CHICAGO

ON THE DECREASE OF PERMEABILITY DUE TO CERTAIN BIVALENT KATIONS

W. J. V. Osterhout

(WITH ELEVEN FIGURES)

It has been shown[1] that while NaCl and many other salts of monovalent metals increase the permeability of protoplasm, $CaCl_2$ has the opposite effect. This effect of $CaCl_2$ is not permanent; if the exposure be sufficiently prolonged, it will be found that it gradually passes away and is followed by an increase of permeability. The question arises, do other bivalent kations behave like calcium?

The method employed in this investigation was to make determinations of the electrical resistance of living tissues of *Laminaria saccharina* in the manner described in a previous paper.[1] Such determinations afford an accurate measure of the permeability of the protoplasm.

The following experiment[2] will illustrate the effects of $CaCl_2$. The resistance of a cylinder of tissue in sea water at 18°C. was found to be 1000 ohms. It was transferred to $CaCl_2$, 0.278M, which had the same conductivity as the sea water. After 15 minutes the resistance in $CaCl_2$ at 18° was found to be 1490 ohms. After 30 minutes more the resistance was still 1490 ohms. Another reading taken 105 minutes later showed the resistance to be 950 ohms; 75 minutes later it was 650 ohms. During this time the control in sea water had not altered its resistance. The results are shown in table I and fig. 1.

The resistance at the beginning of the experiment was 1000 ohms; subtracting from this the resistance of the apparatus (250 ohms) gives the net resistance or the actual resistance of the tissue, which is $1000-250=750$ ohms. The net conductance was $1\div750$

[1] Science N.S. 35:112. 1912.

[2] The chemicals used were the best obtainable and in nearly all cases were Kahlbaum's. The solutions unless otherwise stated were neutral to litmus. This is important, for it has been shown in a previous paper acid may cause a rise in resistance.

=0.00133 mho. After 15 minutes in $CaCl_2$ the net resistance was 1490−250=1240 ohms, and the net conductance 1÷1240=0.00081 mho. We may regard the permeability as equal to the conductivity, or, in this case, for convenience, as equal to the conductance. The decrease in permeability was therefore 0.00133−0.00081=0.00052 mho or 39.1 per cent.

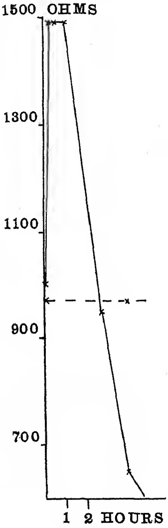

FIG. 1.—Curve of electrical resistance of *Laminaria saccharina* in $CaCl_2$ 0.278 M (unbroken line) and of a control in sea water (dotted line).

TABLE I

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In $CaCl_2$ 0.278 M	In sea water
0	1000	970
$\frac{1}{4}$	1490	970
$\frac{1}{2}$	1490	970
1	1490	970
$2\frac{3}{4}$	950	970
4	650	970

All readings were taken at 15° C.

The characteristic effects of $CaCl_2$ are therefore a very rapid rise followed after an interval by a fairly rapid fall of resistance. It seems probable that these effects result from two processes which go on simultaneously and represent different reactions, one of which has a much greater velocity than the other. In this way the period of stationary resistance (represented by the flattened top of the curve) would be accounted for. The fall in resistance is much slower than that caused by monovalent kations and is, in the opinion of the writer, quite different from it.

Similar results were obtained with $BaCl_2$ and $SrCl_2$.

The behavior of material in a solution of $MgCl_2$, about 0.28 M, having the same conductivity as sea water, is shown in table II and fig. 2. The rise in resistance is not nearly as great as in $CaCl_2$; the fall in resistance begins much sooner and proceeds much more rapidly. The top of the curve is not flattened.

The writer interprets this to mean that the velocity of the second reaction (causing the fall in resistance) is approximately equal to that of the first reaction (causing a rise in resistance).

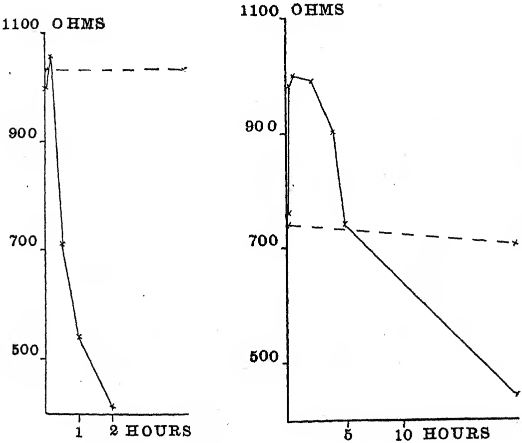

FIG. 2 FIG. 3

FIGS. 2, 3.—Fig. 2, curve of electrical resistance of *Laminaria saccharina* in $MgCl_2$ 0.28M (unbroken line) and of a control in sea water (dotted line); fig. 3, curve of electrical resistance of *Laminaria saccharina* in $MnCl_2$ 0.317M (unbroken line) and of a control in sea water (dotted line).

TABLE II

ELECTRICAL RESISTANCE OF *Laminaria saccharina;* AVERAGE OF THREE EXPERIMENTS

Time in minutes	In $MgCl_2$ 0.28 M	In sea water
0	1000	1030
10	1055	....
30	710	....
60	545	....
120	410	1030

All readings were taken at 18° C.

In a solution of $MnCl_2$ (about 0.317 M), having the same conductivity as sea water, the tissue shows a rapid and very decided rise, followed by a fall which is noticeably slower than that in $CaCl_2$. The results, as shown in table III and fig. 3, are in marked contrast to those obtained with $MgCl_2$.

TABLE III

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In $MnCl_2$ 0.317 M	In sea water
0	760	740
$\frac{1}{4}$	980	...
$\frac{1}{2}$	1000	...
2	990	...
4	900	...
5	740	...
20	440	700

All readings were taken at 18° C.

The rise in resistance in these solutions was so great that it seemed to the writer that a rise might be obtained when the substances in question were added directly to the sea water, either as concentrated solutions or in solid form. Accordingly 10 cc. of $CaCl_2$ 5 M were added to 1000 cc. of sea water and a lot of tissue was placed in it. The resistance rose[3] from 1290 to 1390 ohms, where it remained stationary for a long time and then began to fall. When the same experiment was tried on dead tissue the resistance fell at once and remained stationary indefinitely. The results are shown in tables IV and IVa and fig. 4. The addition of solid anhydrous $CaCl_2$ gave a similar result, although the rise was not as great.

TABLE IV

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. + $CaCl_2$ 5 M 10 cc.	In sea water
0	1290	1320
$\frac{1}{3}$	1280	1320
$\frac{2}{3}$	1300	....
2	1380	1320
$2\frac{1}{3}$	1390	....
13	1390	1320

All readings were taken at 18° C.

[3] The temporary fall in resistance at the start was due to the increased conductivity of the solution contained in the apparatus and in the intercellular substance.

This experiment has a special interest as affording positive proof that the current passes through the protoplasm as well as through the intercellular substance,[4] for it is evident that the rise in resistance in these experiments can not be due to any cause other than a change in permeability of the protoplasm. The concentration of the ions of the sea water remains unchanged, and if they were able to penetrate as freely as they did before the addition of the

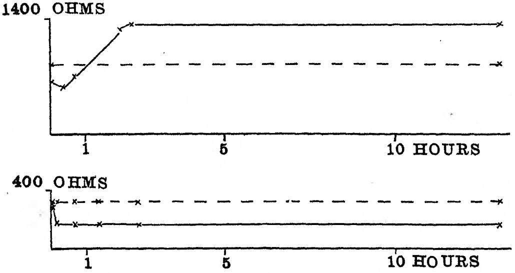

FIG. 4.—Curves of electrical resistance of live tissue (upper figure) and dead tissue (lower figure) of *Laminaria saccharina* in 1000 cc. sea water+10 cc. $CaCl_2$ 5 M (unbroken line in both figures) and of controls in sea water (dotted line in both figures).

TABLE IVa

ELECTRICAL RESISTANCE OF DEAD TISSUE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. + $CaCl_2$ 5 M 10 cc.	In sea water
0	370	380
$\frac{1}{6}$	340	380
$\frac{2}{3}$	340	380
$1\frac{1}{3}$	340	380
$2\frac{1}{2}$	340	380
13	340	380

All readings were taken at 18° C.

$CaCl_2$ the resistance would not increase. It would, in fact, diminish on account of the increased conductivity of the solution held in the intercellular substance, as is clearly shown by experiments on dead tissue. That the change in permeability is in the

[4] The frond may be regarded as a mass of intercellular substance in which the protoplasmic masses are imbedded.

protoplasm and not in the intercellular substance is clearly shown by the fact that as soon as the protoplasm is killed, no rise is produced on adding solid $CaCl_2$. This is true when the means of killing is such as to produce no change in the intercellular substance, e.g., by slight reduction of the water content by partial drying, by allowing the material to stand in the laboratory until dead, or by raising the temperature to 45°C.

If the rise in resistance were not due to a change in permeability, it could be explained only as the result of a decrease in the size of

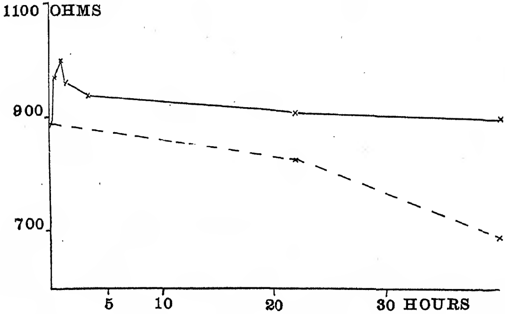

FIG. 5.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+($MnSO_4+7H_2O$) 1.39 gm.=(0.005 M) (unbroken line), and of a control in sea water (dotted line).

the spaces between the cells. Both macroscopic and microscopic measurements show most conclusively that this does not occur. The contrary effect would be produced by the addition of salts in solid form, for they would tend to produce plasmolysis and thereby to increase the space between the cells.

In order to test further the effect of $MgCl_2$, 10 cc. of a 5 M solution were added to 1000 cc. of sea water. A reading taken 5 minutes later showed that the resistance had fallen from 780 to 700 ohms; it continued to fall slowly throughout the experiment. The writer interprets this as showing that $MgCl_2$ is not able to

produce sufficient rise to overcome the effect of the increase in the conductivity of the solution which is contained in the intercellular substance.

The addition of $MnSO_4$ in solid form produced a decided rise as shown in table V and fig. 5. The amount added was sufficient to make the concentration 0.005 M.

TABLE V

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. + $MnSO_4$ 1.39 gm. (=0.005 M)	In sea water
0	890	890
$\frac{1}{2}$	970	...
1	1000	...
$1\frac{1}{2}$	960	...
$3\frac{1}{2}$	940	...
22	910	830
40	900	690

All readings were taken at 18° C.

It is evident that the addition (to sea water) of a salt in solid form is the severest possible test of its ability to produce a rise in resistance. In subsequent tests of other bivalent kations this method was exclusively employed.

In table VI and fig. 6 are shown the results of two experiments with $CaCl_2 + 6\ H_2O$ which was added to sea water in sufficient amount to make the concentration 0.005 M (1.19 gm. to 1000 cc. sea water). The course of the curves is not quite the same, the lower curve falling more rapidly than the upper. This difference is too great to be the result of experimental error and must be attributed to laboratory conditions and to differences in the material itself, which shows some variation in this respect unless gathered and cut at the same time. It will be noticed that the resistance of the control falls off more rapidly in the lower curve, which must be attributed largely to laboratory conditions. Dead tissue under the same conditions showed no rise in resistance.

TABLE VI

ELECTRICAL RESISTANCE OF *Laminaria saccharina:* TWO EXPERIMENTS

Time in hours	In sea water 1000 cc. $(CaCl_2+6H_2O)$ 1.19 gm. (=0.005 M)	In sea water
0	930	890
$\frac{1}{4}$	1010	...
$\frac{1}{2}$	1020	...
1	1010	...
3	980	...
6	960	...
21	940	880
40	900	850
0	940	900
$\frac{1}{4}$	990	...
$\frac{1}{2}$	1020	...
1	1000	...
2	950	...
18	900	...
40	770	780

All readings were taken at 18° C.

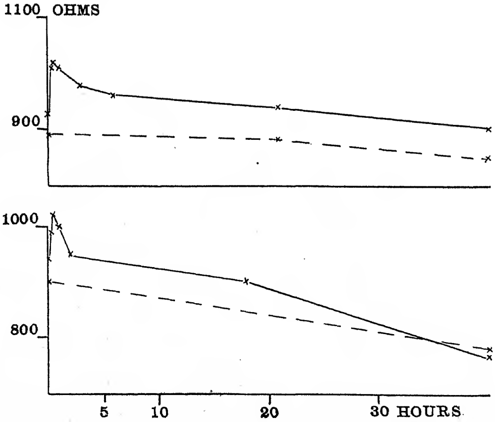

FIG. 6.—Curves of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+$(CoCl_2+6H_2O)$ 1.19 gm. (=0.005 M) (unbroken lines); two experiments; control in sea water (dotted lines).

Table VII and fig. 7 show the behavior of tissue in sea water to which sufficient $FeSO_4 + 7H_2O$ had been added to make the concentration 0.005M (1.39 gm. to 1000 cc. sea water). Some precipitate formed after standing.

TABLE VII

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ($FeSO_4+7H_2O$) 1.39 gm. (=0.005 M)	In sea water
0	910	890
$\frac{1}{12}$	1100	...
$\frac{1}{4}$	1040	...
$\frac{1}{2}$	1000	...
$2\frac{1}{4}$	970	...
18	750	780
40	450	730

All readings were taken at 18° C.

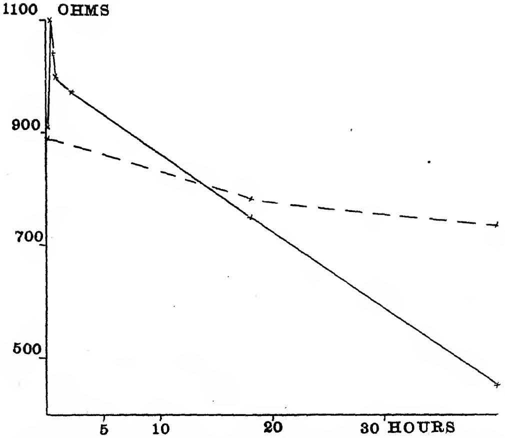

FIG. 7.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc. +($FeSO_4+7H_2O$) 1.39 gm (=0.005 M) (unbroken line) and of a control in sea water (dotted line).

It will be noticed that while there is a very rapid and decided rise, the fall is much more rapid than in any of the previous experiments in which solid salts had been added to sea water. Neither of these effects can be attributed to acid, as the solution was neutral to litmus. After 18 hours the resistance was below that of the control and it continued to fall rapidly to the death point. Dead tissue showed no rise in resistance.

Table VIII and fig. 8 show the results of experiments in which sufficient $NiCl_2 + 6H_2O$ was added to the sea water to make the

TABLE VIII

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ($NiCl_2+6H_2O$) 1.10 gm (=0.005 M)	In sea water
0	940	890
$\frac{1}{4}$	1000	...
$\frac{3}{4}$	1060	...
1	1020	...
2	1000	...
5	950	...
21	920	840
44	760	600

All readings were taken at 18° C.

concentration 0.005 M (1.19 gm. to 1000 cc. sea water). The results are similar to those obtained with $CoCl_2 + 6H_2O$. Dead tissue showed no rise in resistance.

Contrary to expectation the experiments with $ZnSO_4 + 7H_2O$ showed that it was less toxic than $FeSO_4 + 7H_2O$. The results are shown in table IX and fig. 9.

TABLE IX

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ($ZnSO_4+7H_2O$) 1.44 gm. (=0.005 M)	In sea water
0	870	860
$\frac{1}{4}$	940	...
$\frac{1}{2}$	950	...
1	990	...
$1\frac{1}{2}$	970	...
3	920	...
19	830	720
40	700	640

All readings were taken at 18° C.

The concentration used waso .005 M (1.44 gm. to 1000 cc. sea water).

The curve obtained by using $CdCl_2 + 2H_2O$ is of a somewhat different type from those previously met with. The rise is not as

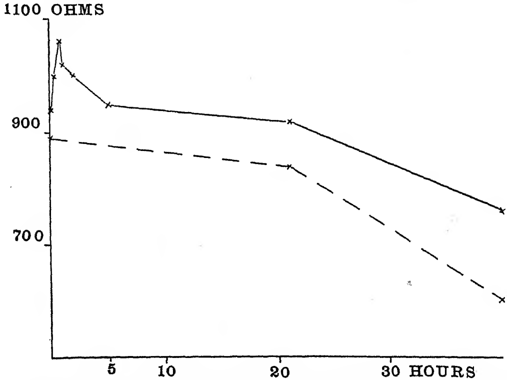

FIG. 8.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+($NiCl_2+6H_2O$) 1.19 gm. (=0.005 M) (unbroken line) and of a control in sea water (dotted line).

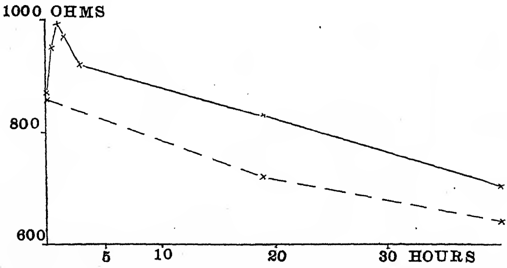

FIG. 9.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+($ZnSO_4+7H_2O$) 1.44 gm. (=0.005 M) (unbroken line) and of a control in sea water (dotted line).

rapid nor as great and the fall is slow, especially at first. The concentration was 0.005 M (1.1 gm. to 1000 cc. sea water). The results are shown in table X and fig. 10. Dead tissue showed no rise in resistance.

TABLE X

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ($CdCl_2+2H_2O$) 1.13 gm. (=0.005 M)	In sea water
0	850	860
$\frac{1}{4}$	900	...
1	920	...
2	930	...
$9\frac{1}{2}$	910	840
$22\frac{1}{2}$	870	820
45	600	680

All readings were taken at 18° C.

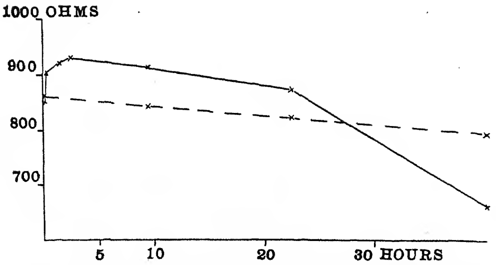

FIG. 10.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+($CdCl_2+2H_2O$) 1.1 gm. (=0.005 M) (unbroken line) and of a control in sea water (dotted line).

A very different type of curve is obtained by using $SnCl_2+2H_2O$. The rise is rapid and decided and the fall is much more rapid than with any of the other substances used. The solution was acid to litmus, but the effect cannot be considered as due to the acidity alone. Dead tissue showed no rise in resistance.

Some precipitate formed on standing. The concentration used was 0.005M (1.13 gm. to 1000 cc. sea water).

TABLE XI

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ($SnCl_2+2H_2O$) 1.13 gm. (=0.005 M)	In sea water
0	780	750
$\frac{1}{4}$	860	...
$\frac{1}{2}$	870	...
$\frac{3}{4}$	880	...
1	830	...
$1\frac{1}{2}$	750	...
17	400	690
40	320	530

All readings were taken at 18° C.

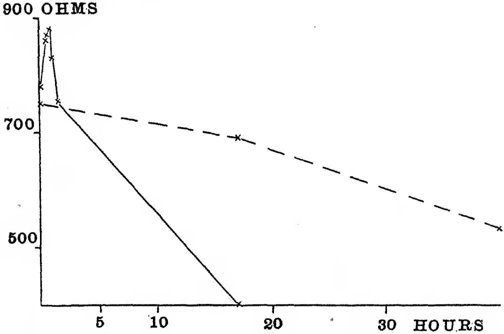

FIG. 11.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+($SnCl_2+2H_2O$) 1.13 gm. (=0.005 M) (unbroken line) and of a control in sea water (dotted line).

It is evident that there is a remarkable difference between the chlorides of monovalent and the chlorides of bivalent kations in their effects on permeability. So far as the writer's experiments have gone, none of the chlorides of monovalent kations are able to decrease permeability (with the significant exception of HCl),

while all of the chlorides of bivalent kations are able to do so to a marked degree. Various tempting hypotheses are suggested by these striking facts. None of them can be worked out at present in a manner which is free from objection, and the writer deems it advisable to defer discussion of them until further investigations have been made.

Summary

There is a remarkable difference between monovalent and bivalent kations in their effects on permeability.

While none of the monovalent kations (except H) are able to decrease permeability, all the bivalent kations so far investigated (Mg, Ca, Ba, Sr, Mn, Co, Fe, Ni, Zn, Cd, Sn) are able to do so to a marked degree.

HARVARD UNIVERSITY

BRIEFER ARTICLES

NOTES ON ORCHIDS

(WITH ONE FIGURE)

CATTLEYA MOSSIAE Hooker.—Mr. KNUDSEN, of Boulder, Colorado, has been very successful in growing this fine species under glass. On one occasion, it was found that the flowers were being fertilized, and it turned out that this was done by *Bombus Huntii* Greene, which gained access to the greenhouse. I now possess one of these bees, with several *C. Mossiae* pollinia attached to the mesothorax. The case is interesting, since this species of bee has had of course no previous experience with *Cattleya* or with any closely related plant. Mr. KNUDSEN believes that honey bees do not pollinate *Cattleya.*

CYTHEREA BULBOSA (L.) House.—On June 8, 1914, my wife and I were able to study this plant in life at Gresham, Colorado. It grew on a damp hillside with a north exposure, under *Populus tremuloides* and young Engelmann spruce, with *Arnica cordifolia* Hook., *Chamaenerion angustifolium* (L.) Scop., and *Fragaria.* We were particularly anxious to see the process of pollination, but in this we were disappointed, owing to the bad weather. We saw no insects on the orchids, but a few *Bombus* were flying around. There can be little doubt that the work is done by *Bombus*, which bending down (almost standing on its head) to get the nectar, would receive the pollen on the upper side of its thorax. In Idaho this orchid is represented by a variety, *Cytherea bulbosa occidentalis* (*Calypso bulbosa occidentalis* Holzinger, Contrib. Nat. Herb. 3:251. 1895), in which the beard on the lip is white instead of yellow. In the Colorado form it is yellow. There is, however, some question whether the Colorado plant is the same as that of the northeastern states, so I give some descriptive details from the living plant as observed at Gresham.

Scape lilac; sepals and petals "similar, ascending, spreading" (AMES), magenta (nearly rose vineaux of GRAVEREAUX, but a little bluer), about 20 mm. long, the median sepal exceeding the lip (wholly different from the figure in BRITTON and BROWN's *Illustrated flora*); sepals and petals 3-veined, but the veins not evident except on close

inspection; winged column exactly the color of sepals and petals, diameter 6.5 mm., apex truncate; lip 19 mm. long, 9.5 mm. broad near base, the basal half profusely streaked with dark crimson on a white ground within, the crimson occupying more than half the surface; at the end of the opening of the lip above is a lemon yellow patch with three rows of yellow hairs, and near the distal end of each row is a bunch of shorter, dark red, claviform hairs; the apical expansion of the lip is about 9 mm. long and 7 mm. wide (longer than in BRITTON'S figure), the apex briefly pointed, not widened or emarginate, as BRITTON'S figure shows; apical part of lip whitish, flushed with pink distally, but without dark spots except the three patches of claviform glands on the yellow area; beneath, the lip or sac ends in two hornlike processes, 2.5–3 mm. long, which do not extend beyond the apical extension above; these horns (which contain nectar) and the region about their base are pale yellowish.

FIG. 1.—*Antholithes pediloides*, n. sp.

CYPRIPEDIUM VEGANUM Cockerell and Barker.—I have grown this successfully in my garden at Boulder, the plants coming from the Upper Pecos. On May 24, 1914, I saw a female bee *Osmia armaticeps* Cresson enter the flower through the upper aperture of the lip, and eventually emerge at the side behind, following the route indicated by H. MÜLLER.[1] It forced its way out with considerable difficulty, the passage being almost too narrow for it. Smaller bees are able to crawl out by the way they came in, and consequently are not agents in pollination.

Antholithes pediloides, n. sp. (fossil).—Lip(?) apparently saccate, as preserved coffee-brown, much darker than the shale, a little over 12 mm. long; no venation visible (fig. 1).

This object, which I have repeatedly studied, has all the appearance of being the lip of a *Cypripedium*, showing a strong callus around the lateral sinuses, and even, by a dark shade, some indication of the margin of the sterile stamen. Comparison with living *Cypripedium* flowers appeared to confirm the identification. On the other hand, it appears very unlikely that a *Cypripedium* lip would be separated from the rest of the flower and preserved in this manner. I think we can say with certainty that the object is neither a fruit nor a leaf; the apparent lateral sinuses are not due to any accidental tearing or breaking. There remains, however, a feature which I cannot at present explain. Irregu-

[1] KNUTH, Blütenbiologie 2:459. 1899.

larly scattered over the surface are small round subhyaline spots, evidently representing perforations of the tissue. These are usually, but not always, in pairs. I suppose that they represent the work of some insect, but what one, I am quite at a loss to imagine.

The fossil was collected by Mr. Geo. N. Rohwer, at Station 14, in the Miocene shales of Florissant, Colorado.—T. D. A. Cockerell, *University of Colorado, Boulder.*

CURRENT LITERATURE

BOOK REVIEWS

A textbook of grasses

We are familiar with books treating grasses from the standpoint of taxonomy or of agriculture, but to make this group the basis of a textbook rather than a book of reference is something unusual. Such a book has been written by HITCHCOCK,[1] and appears as one of "The rural textbook series," under the editorship of L. H. BAILEY. The two parts are "Economic agrostology" and "Systematic agrostology," so that the economic and taxonomic aspects of the group are prominent; nevertheless, the treatment is dominated by the textbook idea, and it is distinctly a teaching book. The titles under "Economic agrostology" are economic classification of grasses, forage plants, cultivated pastures, meadow plants, hay and green feed, lawns, grasses used for miscellaneous purposes, weeds, and grass crop areas. Under "Systematic agrostology" there is a notable textbook introduction under the following titles: morphology of the vegetative organs, morphology of the floral organs, ecology, and principles of classification. This introduction is followed by a taxonomic presentation of the group, which includes a key to all the genera found growing wild or in common cultivation in the United States. The more important genera are described more fully, as they should be, and the principle of selection has been to familiarize the users of the book with the grasses that deserve most attention.

The book will certainly prove very useful to a large and growing constituency, which should include not only rural communities and agricultural colleges, but also students of botany in colleges and universities.—J. M. C.

MINOR NOTICES

Flora of Jamaica.—The third volume of FAWCETT and RENDLE'S *Flora of Jamaica*[2] begins the Dicotyledons. The first volume, published in 1910, included the orchids; while the second volume, not yet published, will include

[1] HITCHCOCK, A. S., A textbook of grasses with especial reference to the economic species of the United States. 8vo. xvii+276. *figs. 63*. New York: Macmillan. 1914. $1.50.

[2] FAWCETT, W., and RENDLE, A. B., Flora of Jamaica, containing descriptions of the flowering plants known from the island. Vol. III. Dicotyledons (Piperaceae to Connaraceae). 8vo. xxiv+280. *figs. 113. pls. 5*. Published by the British Museum. 1914.

the remainder of the Monocotyledons. It is estimated that the Dicotyledons will be completed in four volumes, making the completed work contain six volumes. Preceding the presentation of the families, there is a list of works referred to in the text, a list of names of collectors with the dates of their collections, a conspectus of the families, and a key to families.

The contrast with north temperate floras is striking. Out of 38 families presented, such conspicuous northern ones as Caryophyllaceae (7 spp.), Ranunculaceae (4 spp. of *Ranunculus* and *Clematis*), Cruciferae (6 spp.), and Rosaceae (9 spp.) are negligible elements of the Jamaican flora; while the large families are Urticaceae (55 spp.) and Piperaceae (52 spp.), the largest genera being *Pilea* (42 spp.) and *Peperomia* (38 spp.). The genera number 115 and the species 347, so that, omitting the two large families, the genera average only two species each.—J. M. C.

Flora of New Guinea.—In continuation of the Dutch exploration of the flora of New Guinea, two additional fascicles have appeared.[3] Previous parts were reviewed in this journal.[4]

The fascicle first cited contains the Liliaceae by HANS HALLIER, including 7 genera and 23 species, 8 of which are new; and Piperaceae and Meliaceae by C. DE CANDOLLE. The list of Piperaceae includes 15 species of *Peperomia*, 10 of which are new, and one species of *Piper;* while the Meliaceae are represented by the description of 12 new species.

The second fascicle cited contains the mosses by MAX FLEISCHER, a new genus (*Brotherobryum*) being described in Dicranaceae, and 6 new species in other genera; and Ericaceae by J. J. SMITH, 19 new species being described, mostly in *Vaccinium* and *Rhodendron*. The same author also describes a new *Clethra* and a new *Corsia*.—J. M. C.

Micrography of Javanese woods.—The fourth part of JANSSONIUS' micrography of the woods of Java has appeared,[5] including the Calyciflorae. The previous parts have been noticed in this journal,[6] the first notice describing the general purpose of the work. The present part begins with the Connaraceae and ends with Rhizophoreae. With all the species there are given

[3] Nova Guinea. Résultats de l'expédition scientifique Néerlandaise á la Nouvelle-Guinée en 1907 et 1909 sous les auspices de Dr. H. A. LORENTZ. Vol. VIII. Botanique. Livraison VI. 4to. pp. 989–1048. *pls. 180–188;* the same, L'expédition en 1912 et 1913 sous les auspices de A. FRANSSEN HERDERSCHEE. Vol. XII. Botanique. Livraison II. 4to. pp. 109–172. *pls. 29–54.* Leide: E. J. Brill. 1914.

[4] BOT. GAZ. 49:464. 1910; 55:462. 1913; 57:342. 1914.

[5] JANSSONIUS, H. H., Mikrographie des Holzes der auf Java vorkommenden Baumarten. Vierte Lieferung. 8vo. Vol. III. pp. 1–336. *figs. 183.* Leiden: E. J. Brill. 1914. *M* 6.

[6] BOT. GAZ. 43:345. 1907; 47:616. 1909; 52:67. 1911.

details of the vascular elements, list of reagents, sources of material, references to literature, etc., representing an amount of detailed work that few would care to undertake.—J. M. C.

NOTES FOR STUDENTS

Current taxonomic literature.—O. AMES (Philipp. Jour. Sci. Botany 8:407–440. *pl. 13.* 1913) under the title "Notes on Philippine orchids with descriptions of new species VI" adds 47 new species to the Philippine flora.—E. G. BAKER (Jour. Bot. 52:2, 3. 1914) has published a new genus *Talbotiella*) of the Leguminosae from Nigeria.—H. J. BANKER (Mycologia 5:293–298. 1913) in continuation of studies on the Hydnaceae proposes a new genus (*Hydnodon*) based on *Hydnum thelephorum* Léveille.—G. BEAUVERD (Bull. Soc. Bot. Genève II. 5:205–228. 1913) under the general title "Contribution à l'étude des Composées" describes several new species and proposes the following new South American genera: *Stuckertiella*, based on *Gamochaeta capitata*, and *Berroa*, based on *Lucilia gnaphalioides*.—A. BÉGUINOT (Bull. Soc. Bot. Ital. 97–104. 1913) has published a new genus (*Eremophyton*) of the Cruciferae from Algeria.—A. BERGER (Rep. Sp. Nov. 12:503. 1913) characterizes a new species of *Agave* (*A. Vilmoriniana*) from Mexico.—E. P. BICKNELL (Bull. Torr. Bot. Club 40:605–624. 1913; 41:71–87. 1914) in continuation of the contributions "The ferns and flowering plants of Nantucket" records new species in *Hypericum*, *Crocanthemum*, and *Oenothera*.—G. BITTER (Rep. Sp. Nov. 12:433–467, 542–555. 1913; 13:88–103, 169–173. 1914) in continuation of his studies on the genus *Solanum* records further new species, chiefly from Central and South America. The same author (*ibid.* 12:477–480. 1913) characterizes 5 new varieties of *Polylepis australis* and a new subspecies of *Acaena polycarpa* from Argentina.—A. BRAND (Ann. Conserv. et Jard. Bot. Genève 15 and 16:322–342. 1913) in an article entitled "Neue Beiträge zur Kenntnis der Polemoniaceen" presents important data supplementing his recent monograph of this family and adds several new species and varieties. The same author (*ibid.* 343, 344) describes two new species of *Symplocos* from America, and (Rep. Sp. Nov. 13:81–83. 1914) characterizes two new genera of the Borraginaceae, namely *Lacaitaea*, founded on *Trichodesma calycosum* Collet and Hemsl. of East India, and *Vaupelia* to which are referred six tropical African species hitherto included under *Trichodesma*.—E. G. BRITTON (Bull. Torr. Bot. Club 40:653–676. *pl. 25.* 1913) publishes a preliminary article on West Indian mosses. The author gives rather extended synonomy and bibliography and makes new combinations in *Neckera*, *Clastobryum*, *Palamocladium*, and *Turckheimia*.—E. G. BRITTON and R. S. WILLIAMS (Torreya 14:24–31. 1914) under the heading of "Central American mosses" list 54 species and include a new genus (*Isodrepanium* [Mitt.] E. G. Britton) based on *Homalia lentula* Wils.—L. BUSCALIONI and G. MUSCATELLO (Malpighia 26:1–32, 49–56, 97–144. 1913) in continuation of their studies on the genus *Saurauia* have added three new species from Central and South America.—J. CARDOT (Rev.

Bryol. 40:22, 23. 1913) has proposed a new genus (*Hylocomiopsis*) based on *Anomodon ovicarpus* Besch. of Japan.—T. D. A. COCKERELL (Torreya 13:265–273. 1913) in an article on "Some plants from the vicinity of Longs Peak Inn, Colorado," makes new combinations and records new forms in *Polemonium* and *Senecio*.—O. F. COOK (Contr. U.S. Nat. Herb. 16:277–285. *pl. 101*. 1913) under the title "Nomenclature of the sapote and the sapodilla" gives a brief historical account of these plants and proposes a new generic name for the sapote, namely *Achradelpha mammosa* (L.) Cook.—C. DE CANDOLLE (Coll. of Hawaii Pub. Bull. no. 2. pp. 5–38. *pls. 1–8*. 1913) has published an article entitled "The Hawaiian Peperomias" in which he recognizes 73 species of *Peperomia* of which 47 are new to science.—H. DIEDICKE (Ann. Mycol. 11: 528–545. 1913) in critical notes on certain fungi characterizes a new genus (*Psilosporina*) based on *Psilospora Quercus* Rabenh.—S. T. DUNN (Kew Bull. 145–153. 1913) gives a synoptical revision of the Pacific North American genus *Marah*, recognizing 11 species two of which are new to science.—A. D. E. ELMER (Leafl. Philipp. Bot. 5:1751–1905; and 6:1919–1986. 1913) in cooperation with several specialists has issued articles 93–100, inclusive, which contain descriptions of 166 new species of Philippine plants. The following new genera are proposed: *Schizochora* and *Cyclodothis* Syd. of the Ascomycetes, *Diedickea* Syd., placed in "Fungi Imperfecti," and *Elmerobryum* Broth. of the Hypnaceae.—A. ENGLER (Bot. Jahrb. 51:1–163. 1913) in cooperation with several noted specialists has published the forty-second contribution to the flora of Africa. The families treated here are the Malvaceae, Oleaceae, Violaceae, and Asclepiadaceae. Many new species are described and the following new genera are proposed: *Campanolea* Gilg and Schellenberg of the Oleaceae, *Stigmatorhynchus*, *Blepharanthera*, *Siphonostelma*, and *Kinepetalum* Schlechter of the Asclepiadaceae.—A. J. EWART (Proc. Roy. Soc. Victoria 26:1–11, 152–164. *pls. 1–2, 14–15*. 1913) in cooperation with B. REES and A. MORRISON has issued "Contributions to the flora of Australia, nos. 20, 21." Several new species of flowering plants are described, and one new genus (*Reesia* Ewart) of the Amarantaceae is included.—A. J. EWART and B. REES (*ibid.* 25:105–114. *pls. 5, 6*. 1912) under the title "Contribution to the flora of Australia, no. 19" record several plants as new to or little known in Australia and include a new genus (*Huxleya*) of the Verbenaceae.—K. FRITSCH (Bot. Jahrb. 50:392–439. 1913) under the title "Beitrag zur Kenntnis der Gesnerioideae" has published 34 species new to science and characterizes a new genus (*Fiebrigia*) of the Gesneriaceae from Bolivia.—M. GANDOGER (Bull. Soc. Bot. Fr. IV. 13:454–462. 1913) includes the descriptions of several new species of American flowering plants, particularly in the genus *Polygala*.—P. W. GRAFF (Philipp. Jour. Sci. Botany 8:299–309. *pls. 8–10*. 1913) records important data on the basidiomycetous flora of the Philippines and includes 7 species new to science.—J. M. GREENMAN (Torreya 13:257, 258. 1913) has published a new species of *Senecio* (*S. eriocarphus*) from Cuba.—H. HALLIER (Recueil Trav. Bot. Néerl. 10: 340–355. 1913) characterizes a new genus (*Schuurmansiella*) of the Ochnaceae,

based on *Schuurmansia angustifolia* Hook. f. from Borneo. In the same article the author adds a new species to the genus *Blastemanthus* from Brazil.—R. HAMET (Bot. Jahrb. 50: Beibl. no. 112. pp. 8–12. 1913) has published new species of *Sedum*, including 3 from Peru. The same author (Rep. Sp. Nov. 12:407–411. 1913) adds 3 additional species to this genus from Mexico, and (Field Mus. Nat. Hist. Bot. Ser. 2:378–379. 1913) records two more from Guatemala.—E. HASSLER (Rep. Sp. Nov. 12:365–374, 495–499. 1913) has published several new species, varieties, and forms of Malvaceae, Leguminosae, Anacardiaceae, and Compositae from South America. The same author (Bull. Soc. Bot. Genève 5:266–277. 1913) under the title "Revision critique des Oenothéracées du Paraguay" characterizes several new varieties and forms.—B. HAYATA (Bot. Jahrb. 51:164–176. *pl. 1.* 1913) gives a detailed and very interesting account of the morphology and systematic position of the recently published genus *Mitrastemon*, placing it in the Rafflesiaceae under the tribal name *Mitrastemoneae* which is coordinate with and immediately following the *Apodantheae*.—B. P. G. HOCHREUTINER (Ann. Conserv. et Jard. Bot. Genève 15 and 16:297–303. *pl. 1.* 1913) has published a new genus (*Bakeridesia*) of the Malvaceae from Mexico.—L. S. HOPKINS (Am. Fern. Jour. 3:116–118. *pl. 9.* 1913) describes and illustrates a new species of fern (*Polysticum Andersoni*) from Vancouver Island, British Columbia.—H. D. HOUSE (Torreya 14:2–4. 1914) records a new hybrid violet (*Viola emarginata*×*septemloba*) from southeastern Virginia. The same author (Muhlenbergia 9:81–100. 1914) under the title "Vegetation of the Coos Bay region, Oregon" gives a list of the plants collected during two seasons and includes the description of a new species, namely *Vancouveria brevicula* Greene.—J. HUBER (Bol. Mus. Goeldi 7:199–281. 1913) in an article on the genus *Hevea* adds two hitherto undescribed species from South America. The same author (*ibid.* 283–307) under the title "Sobre una collecçao de plantas da região de Cupaty" describes 15 species new to science and includes a new genus (*Nealchornea*) of the Euphorbiaceae.—O. JIMÉNÉZ (Bol. de Fomento, Costa Rica 3:661–667. 1913) has published an account of a new arborescent fern (*Cyathea gemmifera* Christ) from Costa Rica.—H. JUMELLE and H. PERRIER (Ann. Mus. Col. Marseille III. 1:1–91. *pls. 1–43.* 1913) have published an important contribution to our knowledge of the palms of Madagascar, describing and illustrating several species new to science.—E. KOEHNE (Bot. Jahrb. 51:177–224. 1913) gives a synopsis of the genus *Pygeum*, recognizing 65 species. The article includes several species new to science from the Philippine Islands.—F. KRÄNZLIN (Bull. Jard. Imp. Bot. St. Petersburg 13:89–94. 1913; Rep. Sp. Nov. 13: 117–120, 160, 161. 1914; Kew Bull. pp. 188–192. 1913; Bot. Jahrb. 50: Beibl. no. 112. pp. 1–7. 1913) has published new species of *Buddleia*, several Amaryllidaceae, and other flowering plants from Mexico, Central and South America. The same author (Philipp. Jour. Sci. Bot. 8:311–33. 1913) under the title "Cyrtandraceae novae philippinenses II" has published 34 new species mostly of the genus *Cyrtandra*.—G. KÜKENTHAL (Rep. Sp. Nov. 13:135, 136.

1914) describes several new Cyperaceae including a new species of *Rhynchospora* (*R. Ostenii*) from Uruguay.—A. E. Lechmere (Bull. Soc. Myc. France **29**:303–331. *pls. 20, 21.* 1913) under the title "Description de quelques Moisissures nouvelles provenant de la Côte d'Ivoire" characterizes a new genus (*Peristomium*) of the Sphaeriaceae from French Congo.—G. Lindau (Rep. Sp. Nov. **12**:423–426. 1913) has published 5 new species of Acanthaceae from Central America.—Th. Loesener (Verh. Bot. Ver. Prov. Brandb. **55**:151–194. 1913) in cooperation with several specialists has published the eighth article under the general title "Plantae Selerianae." Several species new to science are described.—J. Lunell (Am. Mid. Nat. **3**:135–140. 1913) under the title "*Rosa* in North Dakota" includes descriptions of three new species, and (*ibid.* 141–148) characterizes several new species and varieties of flowering plants from North Dakota and a new *Rhus* from Nebraska.—K. K. Mackenzie (Bull. Torr. Bot. Club **40**:529–554. 1913) continues "Notes on *Carex*" and gives a key to the *Montanae* group, recognizing 14 species of which 5 are designated as new. The same author (Torreya **14**:67, 68. 1914) has described a new genus (*Geocarpon*) of the Aizoaceae.—R. Maire (Ann. Mycol. **11**:331–358. *pls. 16–18.* 1913) under the title "Études mycologiques" includes descriptions of the following new genera: *Amanitella*, *Rhodopaxillus*, and *Periolopsis*.—G. O. Malme (Arkiv för Botanik **13**: no. 3. pp. 1–103. 1913) under the title "*Xyris* L. Untergattung *Nematopus* (Seubert), Entwurf einer Gleiderung" presents a synopsis of the group and describes several new species from South America.—S. C. Mason (Jour. Agr. Research **1**:147–178. *pls. 9–16.* 1913) describes and illustrates several hybrids and a new species of *Prunus* from southwestern United States.—E. D. Merrill (Philipp. Jour. Sci. Botany **8**:31–63. *pl. 1.* 1913) under the title "Studies on Philippine Rubiaceae I" includes descriptions of 35 new species. The same author (*ibid.* 335–360. *pls. 11, 12*) in a second paper on "Philippine Melastomaceae" presents a consideration of the tribe *Astronieae*, describing 14 new species in *Astronia*, one in *Beccarianthus*, and proposes a new monotypic genus *Everettia* (*E. pulcherrima*); also (*ibid.* 363–390) under the title "Plantae Wenzelianae" includes diagnoses of 28 new species of flowering plants, collected near Dagami, Leyte, P.I.—G. K. Merrill (Ottawa Nat. **27**:117–121. 1913) records several new and noteworthy lichens from Vancouver Island and the Rocky Mountains.—C. Mez (Rep. Sp. Nov. **12**:411–421. 1913) has published new species of Bromeliaceae from the West Indies and South America.—C. F. Millspaugh (Field Mus. Nat. Hist. **2**:353–377. 1913) presents the results of studies in the Euphorbiaceae and gives a synoptical revision of *Pedilanthus*, recognizing 31 species of which 9 are designated as new. *Cubanthus* which was treated by Boissier as a section under *Pedilanthus* is raised to generic rank and two species are referred thereto. One new genus (*Dendrocousinia*) is characterized from Jamaica.—C. E. Monroe (Bull. Wis. Nat. Hist. Soc. **11**:74–105. 1913) has published a paper on the "Wild asters of Wisconsin" and includes a catalogue of 60 recognized species and varieties.—G. V. Nash (Torreya **13**:273–

274. 1913) describes two new species of American grasses.—J. A. NIEUWLAND (Am. Mid. Nat. 3:170–197. 1914) under the title "Critical notes on new and old genera of plants I" proposes several new generic names and makes a number of new combinations.—J. A. NIEUWLAND and R. M. KACZMAREK (*ibid.* 207–217. 1914) present a generic segregation of *Viola*.—I. OCHOTERENA (Mem. Soc. Alzate 33:93–113. *pls. 6–21.* 1913) under the title "Plantas Deserticas Mexicanas" describes and illustrates several species of *Agave* and *Yucca*, including the following new to science: *A. complicata* and A. *quiotifera* Trelease.—E. PALLA (Oesterr. Bot. Zeitschr. 63:401–404. 1913) has published 5 new species of Cyperaceae from Mexico.—C. H. PECK (N.Y. State Mus. Bull. 167. pp. 1–52. *pls. 131, 132*, ix, x. 1913) records important data concerning the flora of New York and includes descriptions of several new species of fungi. —H. PITTIER (Sm. Misc. Coll. 63: no. 4. pp. 1–4. 1914) in an article on the "Relationship of the genus *Aulacocarpus*" has described a new species (*A. completens*) from Panama.—L. QUEHL (Monats. für Kakteenkunde 23:181. 1913) has published a new species of *Mamillaria* (*M. arida*) from Lower California.—L. RÀDLKOFER (Philipp. Jour. Sci. Bot. 8:443–473. 1913) gives an enumeration of the Sapindaceae of the Philippine Islands, recording 116 species of which 27 are new to science. Two new genera are proposed, namely *Gleocarpus* and *Gongrospermum*.—H. REHM (*ibid.* 391–405) in a third article on Philippine Ascomycetes includes descriptions of 24 new species and several varieties.—J. F. ROCK (Coll. Hawaii Pub. Bull. no. 2. pp. 39–49. *pls. 9–12.* 1913) under the title "New species of Hawaiian plants" has described new species and varieties of flowering plants.—R. A. ROLFE (Kew Bull. 1913. pp. 141–145, 338–343) has published new species of orchids including 7 from Central and South America.—J. N. ROSE (Sm. Misc. Coll. 61:no. 12. pp. 1, 2. *pl. 1.* 1913) records a new poplar (*Populus MacDougalii*) from the southwest.—E. ROSENSTOCK (Rep. Sp. Nov. 12:468–477. 1913) has published several species and varieties of ferns from Bolivia based on collections of Dr. O. BUCHTIEN.—H. ROSS (Mem. Soc. Alzate 32:155–199. *pls. 11–13.* 1913) in cooperation with specialists has published under the heading "Contributions à la flore du Mexique" a list of plants obtained on excursions in connection with the Tenth International Geological Congress held in Mexico City in 1906. The present paper concerns the groups from lichens to lycopods inclusive, records important data, and includes a description and illustration of a new fern (*Dryopteris Rossii*).—P. A. SACCARDO (Ann. Mycol. 11:312–325, 546–568. 1913) under the title "Notae mycologicae" has published numerous new species of fungi and proposes the following new genera: *Haraea* and *Actinopelte* from Japan, and *Melanographium*, *Traversoa*, and *Stigmatomyces* from the Philippine Islands.—P. A. SACCARDO and A. TROTTER (*ibid.* 409–420) in an article entitled "Fungi Tripolitani" include a description of a new hymenomycetous genus *Lacellina*.—C. S. SARGENT (N.Y. State Mus. Bull. no. 167. pp. 53–124. 1913) gives a synopsis of the genus *Crataegus* as represented in New York, recognizing 219 species of which 26 are described as new.—H.

SCHENCK (Rep. Sp. Nov. 12:360–363. 1913) has published 10 new species of *Acacia* from Mexico and Central America.—R. SCHLECHTER (*ibid.* 481–495) describes 30 new species of orchids from Bolivia. The same author (Ann. Mus. Colon. Marseille III. 1:148–202. *pls. 1–24.* 1913) contributes an important article on the orchids of Madagascar, describing and illustrating about 50 species new to science.—M. SLOSSON (Bull. Torr. Bot. Club 40:687–690. *pl. 26.* 1913) has described a new *Trichomanes* from Colombia and a new *Polystichum* from Cuba.—W. W. SMITH (Rec. Bot. Surv. Ind. 4:323–431. 1913) under the title of "The alpine and subalpine vegetation of Southeast Sikkim" gives a detailed account of a botanical survey of this region and lists 925 species of plants. Several species new to science are included, and one new genus (*Paroxygraphis*) of the Ranunculaceae is characterized.—O. STAPF (Kew Bull. 1913. pp. 354, 356) has published two new genera (*Chlamydoboea* and *Dichiloboea*) of the Gesneriaceae from tropical Asia.—J. STUCHLIK (Beih. Bot. Centralbl. 30^2:392–411. 1913) under the heading "Über einige neue Formen von *Gomphrena*" records several new forms of this genus from America. The same author (Rep. Sp. Nov. 12:516–524. 1913) in continuation of his studies on *Gomphrena* describes additional new varieties and forms.—D. R. SUMSTINE (Mycologia 6:32–36. *pls. 115–117.* 1914) under the title "New or interesting fungi" describes new species and proposes a new genus (*Hormisciopsis*).—W. T. SWINGLE and M. KELLERMAN (Jour. Agr. Research 1:419–436. *pl. 49.* 1914) have described a new genus (*Citropsis*) of the Rutaceae and have transferred thereto five species. The genus is based upon *Limonia Preussii* Engler, a species native of Kamerun which seems to possess economic possibilities.—H. and P. SYDOW (Philipp. Jour. Sci. Bot. 8:475–508. 1913) continue their enumeration of the Philippine fungi and characterize the following new genera: *Bulgariastrum*, *Calopeziza*, *Sirosphaera*, and *Lasiothyrium*. The same authors (Ann. Myc. 11:402–408. 1913) under the heading "Novae fungorum species XI" describe several new species and propose the following genera: *Micropeltella*, based on *Micropeltis albomarginata* Speg. from the Philippine Islands, and *Petrakia*, based on *Epicoccum echinatum* Pegl. from Austria.—F. THEISSEN (*ibid.* 425–467. *pl. 20;* 493–511. *pl. 21.* 1913) has published several new species of South American fungi and characterizes the following new genera: *Amazonia*, *Thallochaete*, *Myxomgriangium*, and *Heterostoma* from Brazil; also *Morenoina*, based on *Morenoella antarctica* Speg., and *Lembosina*, based on *Lembosia copromya* B. R. S.—C. TORREND (Broteria Ser. Bot. 11:165–181. 1913) under the title "Troisième contribution pour l'étude des champignons de l'ile de Madère" continues the enumeration of species including several new to science and proposes a new genus (*Menezesia*) of the Protomycetaceae.—W. TRELEASE (Ann. Conserv. et Jard. Bot. Genève 15 and 16:351. 1913) describes a new species of *Phoradendron* from Colombia.—E. ULBRICH (Verh. Bot. Ver. Prov. Brandbg. 55:50–54. 1913) has published a new genus (*Selera*) of the Malvaceae, related to *Gossypium*, from Mexico.—I. URBAN (Rep. Sp. Nov. 13:152–159. 1914) records the results of further study in the

Turneraceae describing new species in *Piriqueta* (2) and *Turnera* (3) from South America.—H. F. WERNHAM (Jour. Bot. 51:320–324. 1913) under "New Rubiaceae from Tropical America III" has published 10 new species.—Ö. WINGE (Arkiv för Botanik 12: no. 9. pp. 1–39. *pls. 1–13.* 1913) under the title "Cytological studies in the Plasmodiophoraceae" includes a description of a new genus and species (*Sorodiscus Callitrichis* Lagerh. and Winge) found on stems of *Callitrichis vernalis.*—H. W. WOLLENWEBER (Phytopathology 3: 197–242. *pls. 20–22.* 1913) characterizes a new genus (*Cylindrocarpon*) based on *Nectria cucurbitula* (Tode) Fries.—N. WORONICHIN (Monit. Jard. Bot. Tiflis, Livr. 28. pp. 16–25. 1913) has published several new species of parasitic fungi including a new genus (*Echinosporium*) found on leaves of *Acer Pseudoplatanus* L. in the Caucasus region.—K. YENDO (Nyt Mag. f. Naturv. 51:275–288. *pls. 13, 14.* 1913) under the title "Some new algae from Japan" describes and illustrates a new genus and species of parasitic alga, namely *Benzaitenia yenoshimensis.* The same author (Trav. Mus. Bot. and Acad. Imp. Sci. St. Pétersbourg 10:114–121. 1913) presents a discussion of *Haplosiphon filiformis* Rupr. and proposes a new genus (*Ruprechtiella*), based on a part of RUPRECHT's material in the Herbarium of the Academy of Science in St. Petersburg.—J. M. GREENMAN.

Tree growth.—Measuring the growth in height of over 40 specimens of *Pinus ponderosa* for the five years of 1909–1913, and attempting to correlate these increments with the precipitation during the growing period, KIRKWOOD[7] concludes that the amount of growth is determined principally by the moisture conditions of the preceding growing season. This he shows to be directly in harmony with the fact that the time of increase in height and thickness in trees is limited to the first few weeks of the growing season. His quantitative data seem to warrant such a conclusion, at least for regions of limited rainfall, where the seasons of maximum precipitation would approach most nearly to the optimum requirements of the tree.

Investigations of a somewhat similar nature by JACCARD[8] include observations upon a small number of individuals of several different species during the growing seasons of 1911 and 1912. The former was the warmer, drier year, and showed a greater increase in thickness in three examples compared with six examples exhibiting the greater increment in the latter year. He also shows that the period in which most of the increase in size is accomplished is during the first half of the season, or more exactly from May until the middle of July. He makes no attempt to trace the connection between the growth of one season with the weather conditions of the previous year.

[7] KIRKWOOD, J. E., The influence of the preceding growing season on the growth of the yellow pine. Torreya 14:115–125. 1914.

[8] JACCARD, P., Accroisement en épaisseur de quelques conifères en 1911–1912. Jour. Forestier Suisse, nos. 6, 7, 8. pp. 1–20. 1913.

In a more extensive investigation, KAPTEYN[9] regards it important that the data of tree growth, to be reliable, should be from trees in rather extensive forests, well situated with respect to subsoil water, and where the conditions over considerable areas are uniform. His own data were derived from annual ring measurements of oaks taken from the forests along the rivers Main, Moselle, and Rhine, and include the increments for the past two centuries. During this period the fluctuations in growth rate showed parallel variations in the three forests, and these variations correlated with meteorological records lead him to the conclusions that: (1) the very considerable fluctuations in the yearly growth of the oaks in the forests under consideration must, in large part, be due to meterological influences; (2) temperature has had a very small influence; (3) the rainfall of the spring and summer is the factor of the most importance, but its influence may be different for different kinds of trees; (4) increased growth seems to be caused by a greater supply of subsoil water rather than by any more direct action of greater precipitation; (5) for at least the last 70 years of the period there was but a single growth ring produced each year; (6) there appears to be a rather constant periodicity of 12.4 years in the rate of growth of these trees, and a comparison with some specimens of *Sequoia* from California would indicate a similar periodicity in their annual increment.

All these papers are suggestive rather than conclusive in their results, and indicate the importance of more extensive data before very definite conclusions can be reached.—GEO. D. FULLER.

First-generation maize hybrids.—COLLINS[10] has described a method of comparing the yield of first-generation hybrids between distinct varieties of maize with the yield of the parent varieties. The principal difficulties with methods heretofore in use are thought to have arisen from failure to appreciate (1) the importance of individual diversity in such hybrids as well as in the parent varieties, (2) the abnormal behavior of self-pollinated maize plants, and (3) the necessity of securing for the comparison hybrids and parents of identical ancestry. Briefly, the method suggested for obtaining the material for comparison is to select two plants, *1* and *2*, from each of two varieties, *A* and *B*, and by hand-pollination to make the four combinations represented by $A1 \times A2$, $A2 \times B1$, $B1 \times B2$, and $B2 \times A1$, resulting in one cross-pollinated ear of each variety and two ears representing the hybrid between the varieties. The reviewer does not doubt that, if a considerable number of these sets of four ears were similarly obtained, the method would afford an accurate means of comparing the yields of maize varieties as they exist with the yields of first-generation crosses between these varieties, and that it should therefore be of

[9] KAPTEYN, J. C., Tree growth and meteorological factors. Rec. Trav. Bot. Néerland. 11:70–93. 1914.

[10] COLLINS, G. N., A more accurate method of comparing first-generation maize hybrids with their parents. Jour. Agric. Research 3:85–91. 1914.

no little value in practical agronomic tests. But he is not prepared to accept the author's idea that the proposed method affords a reliable means of "measuring the effect of crossing apart from other factors that influence yield." The method does not afford a comparison between the hereditary yielding power (effect of genetic factors influencing yield) on the one hand, and on the other the effect of these same genetic factors plus the effect of crossing (heterozygosis?). Since pronounced individual diversity exists in all ordinary maize varieties, the comparison offered is in reality between (1) the effect of certain genetic factors plus the effect of crossing between somewhat unlike individuals (an unknown degree of heterozygosis ?), and (2) the effect of the same genetic factors plus the effect of crossing between individuals presumably, though not necessarily, more unlike (a presumably considerable though wholly unknown degree of heterozygosis ?).—R. A. EMERSON.

Aspen in reforestation.—Experimental evidence is presented by PEARSON[11] of the extent to which the aspen assists in reforestation by promoting the vigor of conifer seedlings. The experiments were conducted by comparing the survival and condition of young Douglas fir (*Pseudotsuga Douglasii*) planted on similar areas with and without aspen cover, the results being decidedly better in the former localities. Measurements of the evaporating power of the air in the two situations show it to be decidedly less among the aspens, and to this is ascribed the better success of the young Douglas firs. Data upon soil moisture are less convincing, particularly from the absence of any constant, such as the wilting coefficient, to determine the availability of the moisture which is present. Incidentally, attention is directed to the importance of vegetative reproduction in the establishment of the aspens.—GEO. D. FULLER.

Sporophyte of liverworts.—Using the sporophyte of Hepaticae as a basis of classification, DOUIN[12] would make three groups as follows: those with the sporophyte reduced to a capsule (Ricciales); those with foot and capsule only (Anthocerotales); and those with foot, seta, and capsule (all of the rest of the liverworts). Although regarding the Anthocerotales as a very natural group, he objects strongly to making them coordinate with Hepaticae and Musci. The reviewer's recent studies of Mexican and Polynesian Anthocerotales, especially a form from Samoa, most emphatically bear out DOUIN's view. DOUIN concludes that the Jungermanniaceae Acrogynae, although now divided very artificially by various writers, are a far more natural assemblage than are the Anacrogynae, which as now arranged are the most artificial assemblage among Hepaticae.—W. J. G. LAND.

[11] PEARSON, G. A., The rôle of the aspen in the reforestation of the mountain burns in Arizona and New Mexico. Plant World **17**:249–260. 1914.

[12] DOUIN, ROBERT, Le sporophyte chez les Hepatiques. Rev. Gén. Botanique **24**: 403–413, 453–463. *pls. 18–21.* 1912.

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THE

BOTANICAL GAZETTE

MAY 1915

BRANCHING IN THE OPHIOGLOSSACEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 202

LOREN C. PETRY

(WITH PLATES XX AND XXI AND SIX FIGURES)

The occurrence of branching of the rhizome in this family was mentioned by ROEPER (13) in 1859, when he described and figured specimens of *Botrychium Lunaria* in which the rhizome bore lateral branches. The origin of such branches was investigated by BRUCHMANN (2), who concluded that they were from adventitious buds of superficial origin. FARMER and FREEMAN (4) had already ascribed the occasional monopodial branching of *Helminthostachys* to the occurrence of adventitious buds. In 1902, GWYNNE-VAUGHAN (5) pointed out that small conical masses of parenchyma occur regularly in the axils of the leaves in this genus and suggested that these are vestigial buds. This view has been confirmed by LANG (8), who found that the branches are always axillary in position. The same writer (9) has also shown the regular occurrence of similar vestigial buds in the axils of the leaves in *B. Lunaria*, and has demonstrated that the branches of the rhizome arise from these, and not from buds of adventitious origin, as stated by BRUCHMANN.

A branching rhizome of *Ophioglossum vulgatum* was figured by STENZEL (14) in 1858; although no statement was made, the figure clearly indicates that the branching is dichotomous. VAN TIEGHEM (15) reported similar specimens and, disregarding ROEPER'S figures, stated that all branching of the rhizome in this family is

dichotomous. This view of the character of the branching was confirmed for *O. vulgatum* by POIRAULT (11).

The occurrence of the two methods of branching within this family has suggested the desirability of further examination of this feature. This investigation has accordingly been undertaken with a view to securing further data bearing upon the relation of the three genera to each other and of the family to other Pteridophytes.

Ophioglossum

POIRAULT (11) made the first critical examination of the nature of the branching of *O. vulgatum*. He concluded that the branching is usually only apparent, and that it is due to the development of a stem bud upon a young root before the root has broken through the

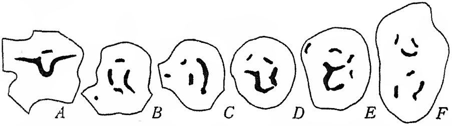

FIG. 1.—Transverse sections of a branching rhizome of *Ophioglossum vulgatum;* only the xylem is shown; ×7.

cortex of the parent rhizome. In a single instance true branching was found, and anatomical examination showed this to be dichotomy. So far as the writer is aware, there is no record of branched rhizomes in other species of this genus.

Ophioglossum vulgatum.—In the examination of some 300 specimens of this species, five branched rhizomes were found. These were examined in serial sections, and in all cases the original stele has divided into two equal and similar steles (fig. 1). There can be no doubt that this is dichotomy, as contrasted with monopodial branching. The term "dichotomy" is here used to denote that branching in which a stem divides into two equal stems, as in *Lycopodium*, and is not meant to imply an exactly equal division of the apical cell of the rhizome.

Ophioglossum pendulum.—In the examination of some 100 rhizomes of this species, two branched specimens were found. As

in *O. vulgatum*, the branching is dichotomous, and the two branches in each case showed an almost exact equality.

In the examination of the material of these two species of *Ophioglossum*, careful search was made for evidence of axillary buds, such as have been described for *Helminthostachys* by GWYNNE-VAUGHAN (5), and for *Botrychium Lunaria* by LANG (9). No such structures have been found in any case; neither is there any evidence of adventitious budding except upon roots. From this it may be concluded that the true branching of these species is always dichotomous.

Helminthostachys

LANG (8) has briefly described the anatomy of two branching rhizomes of this genus. In both, the branches are definitely axillary in position, and it is certain that they have developed from the vestigial buds described by GYWNNE-VAUGHAN (5). In each case, the vascular supply of the branch comes from a mass of accessory xylem which develops outside the usual xylem of the stele, either locally at the base of the branch, or surrounding the stele. There is no connection between the branch stele and the subtending leaf trace.[1]

Material of *Helminthostachys* has not been available during the progress of this investigation.

Botrychium

THE OCCURRENCE OF AXILLARY BUDS

All the investigation of branching and of the occurrence of buds has been confined to examination of *B. Lunaria*. On this account, it has seemed advisable to examine other species of this genus with regard to these points. Rhizomes of five species have been secured with branching specimens representing four of these species.

The genus *Botrychium*, as organized by PRANTL (12), consists of two sections: EUBOTRYCHIUM, with five species; and PHYLLOTRICHIUM, with eleven species. The latter section is divided into

[1] The full description of this material has just been published (LANG, WM. H., Studies in the morphology and anatomy of the Ophioglossaceae. III. On the anatomy and branching of the rhizome of *Helminthostachys zeylanica*. Ann. Botany **29**: 1–54. *pls. 1–3. figs. 1–8.* 1915). In this paper LANG concludes that although no regular cambium is present, "the development of this accessory xylem should rightly come under the conception of secondary thickening."

two subsections: TERNATA, of eight species; and CICUTARIA, of three species. The material investigated consists of rhizomes of *B. ramosum* and *B. lanceolatum* var. *angustisegmentum*, of the section EUBOTRYCHIUM, to which *B. Lunaria* also belongs; of *B. obliquum* and *B. ternatum* var. *intermedium*, of the subsection TERNATA; and of *B. virginianum*, of the subsection CICUTARIA.

Rhizomes of these five species were examined in serial sections for the presence of such axillary buds as have been described by

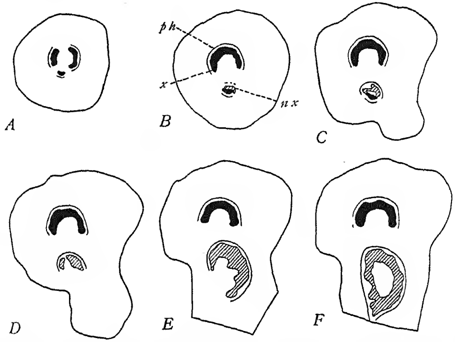

FIG. 2.—Transverse sections of a branching rhizome of *Botrychium lanceolatum* var. *angustisegmentum*: *x*, original xylem of the stem and leaf trace; *nx*, xylem formed after the injury; *ph*, phloem; ×12.

LANG. In all these species, such buds are uniformly present in rhizomes of all ages, and it may be assumed that they are of constant occurrence in all species of the genus. Their origin and development will be considered later.

VASCULAR CONNECTIONS OF THE BRANCHES

ROEPER'S (13) figures represent rhizomes of *B. Lunaria* with 2–7 lateral branches. HOLLE (6) figured a single branching specimen of the same species in longitudinal section, and pointed out that in this case the branch stele connects with the trace of the leaf

immediately below. LANG (9) has given in detail the vascular connections of the branches in five specimens of this species; he concluded that "the chief vascular supply of the branch is derived from a development of xylem adaxially to the subtending leaf trace." This adaxial xylem is considered to be an extension of the margins of the leaf trace, and therefore centrifugal in character.

Botrychium lanceolatum var. *angustisegmentum*.—A single branching specimen of this species was secured. The terminal bud has been destroyed and the branch has arisen at a considerable distance below. As shown by fig. 2, the vascular system of the branch connects with the subtending leaf trace. After the trace has been separated from the stem stele for a considerable distance, tracheids appear on the adaxial side of the protoxylem of the trace; as shown by fig. 3, these are definitely centripetal in origin and occur in contact with the protoxylem. The mass of xylem formed in this way gives rise to the branch stele, which soon assumes the characters of the main stele. This differs from the general condition in *B. Lunaria*, as described by LANG, in that the branch connection arises from centripetal xylem of the leaf trace rather than from adaxial extension of the centrifugal xylem.

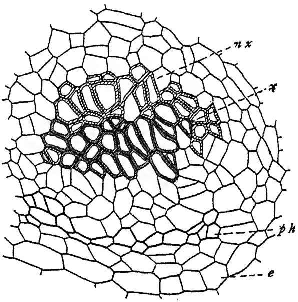

FIG. 3.—Detail of leaf trace of fig. 2, *B*: *e*, endodermis; ×157.

Botrychium ramosum.—Two rhizomes of this species, each bearing a single branch, were secured. In each case the terminal bud has been destroyed and the branch has developed at a considerable distance below. The vascular connections of the branches differ greatly from that described above.

Fig. 4 shows the vascular supply of the branch in the first of these specimens. As the leaf trace swings away from the stem stele, wings of primary and secondary xylem develop in the angle

between the two (figs. 4, *B*, and 7). At first these extend the entire distance between the two and give the appearance of expansion of the stele in the plane of the leaf trace; but later they separate from the stem stele, as shown by fig. 4, *C*. These wings may be considered to be adaxial extensions of the centrifugal xylem of the leaf trace; there is at no point any evidence of centripetal xylem. Soon after the separation from the stem stele, the leaf trace disappears, having been cut off by the absciss layer and carried out by periderm formation. This leaves two distinct wings of xylem and *each* of these

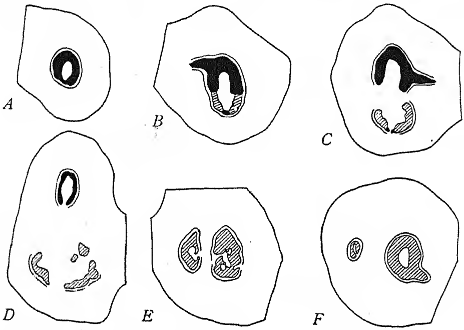

FIG. 4.—Transverse sections of a branching rhizome of *Botrychium ramosum*; ×12.

by extension of its margins rounds up into a definite branch stele, as shown in figs. 4, *D*, *E*, *F*, and 8. One of these branch steles soon disappears, having apparently been unable to meet the competition; its apical region has been entirely obliterated by periderm formation. There can be little doubt that both these branch steles developed from a single axillary bud.

In the other branching specimen of this species, a wing of xylem extends between the leaf trace and the stem stele on one side only. It separates from the stem stele and at a slightly higher point the leaf trace disappears. By extension of its margins, the

wing of xylem rounds up to form a single branch stele. This is exactly the behavior of each of the two wings in the first specimen.

The vascular supply of the branch is here derived in part, at least, from adaxial extension of the centrifugal xylem of the leaf trace, as in *B. Lunaria;* but the formation of either one or two wings of xylem and the consequent formation of one or two branch steles is unique. The significance of this will be discussed later.

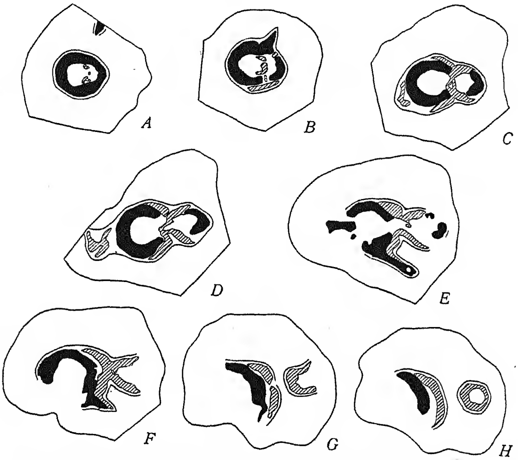

FIG. 5.—Transverse sections of a branching rhizome of *Botrychium virginianum;* ×7.

Botrychium virginianum.—Two branching rhizomes of this species were examined; one of these bore two branches, the other a single one. Fig. 5 shows the vascular connections of the branch in one of these; the injury occurred at the side of the stem and destroyed almost the entire stele (fig. 5, *G*, *H*). As indicated by the figure, there is a development of xylem in the space between the outgoing leaf trace and the stem stele, as in *B. ramosum;* but

here both wings contribute to the formation of a single branch stele. Also procambium strands arise in the pith of the stem opposite the leaf trace (fig. 5, *A*, *B*); these develop a cambium directed toward the center of the stem, which produces a small amount of centripetal secondary wood which also contributes to the vascular supply of the branch. But the greater part of the vascular supply is furnished by the extra or accessory secondary xylem produced by the renewed activity of the cambium (fig. 5, *C*). In this case, therefore, the branch connection consists of (1) accessory secondary xylem, (2) adaxial extensions of the centrifugal xylem of the leaf trace, and (3) a small mass of centripetal secondary xylem originating within the pith of the leaf gap.

One of the other branches shows almost exactly the same connection, but the third branch shows a somewhat different situation. In it the vascular supply is composed principally of accessory secondary xylem; but a small mass of xylem appears in the pericycle of the stem and contributes to the supply of the branch. This pericyclic xylem is added to by a cambium directed toward the phloem; hence we may speak of primary and secondary pericyclic xylem, using the terms "primary" and "secondary" only to indicate the presence or absence of a definite cambium. The vascular supply of this branch, therefore, consists of (1) accessory secondary xylem and (2) primary and secondary pericyclic xylem.

Botrychium obliquum.—Four branching rhizomes of this species were secured; three of these bore two branches each, and the other had three branches. In all but one of these, the apical region had been destroyed; but in this one case the rhizome had been injured at the side below the apical region, but without destroying the entire stele. In all the other cases, the branches developed near the apical region. The vascular connection of the lowest of the three branches mentioned above is shown by fig. 6.

As shown by fig. 6, *A*, a very large development of accessory secondary xylem occurs entirely around the stele before the leaf trace separates; at the same level, a cambium has formed within the pith opposite the leaf trace and has developed a considerable mass of centripetal secondary xylem. The injury which occurred at the side of the stele at a slightly higher level has resulted in the

destruction of the greater portion of the original xylem of the stem; the attendant periderm formation has produced a considerable distortion of the stele. As the leaf trace separates from the stele (fig. 6, *C*) it is surrounded by a ring of xylem composed on the outer side of accessory secondary wood and on the inner side of centripetal secondary wood. This ring splits into two masses (fig. 6, *D*), and the leaf trace is cut off and carried out by periderm

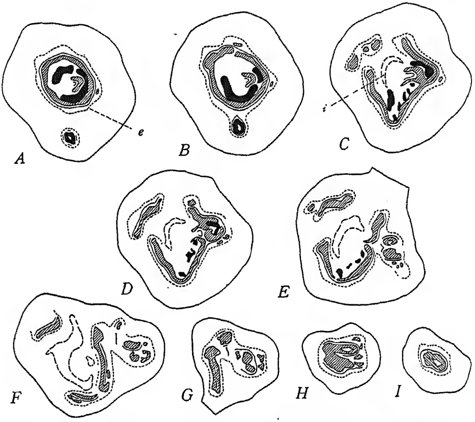

FIG. 6.—Transverse sections of a branching rhizome of *Botrychium obliquum*: *i*, injured region; ×3.5.

formation. At a somewhat higher level, the accessory secondary xylem closes across the gap (fig. 6, *F*).

At a lower level (fig. 6, *E*), masses of xylem arise in the pericycle of one of the strands which formed part of the ring surrounding the leaf trace. Similar masses of pericyclic xylem occur outside the accessory secondary wood which closed the leaf gap (fig. 6, *F*). The development at this point results in the formation of a closed ring of xylem with cambium and phloem on the inside (fig. 6, *G*); at a slightly higher level, this becomes a tangled mass of tracheids

with occasional scattered sieve tubes and parenchyma cells. All these strands—the two produced by the splitting of the ring of xylem about the leaf trace, the strands which arise in the pericycle of one of those, and the tangled mass which is both accessory and pericyclic in origin—fuse to form the branch stele (fig. 6, *H*). Hence the vascular supply of the branch consists of (1) centripetal secondary xylem, (2) accessory secondary xylem, and (3) primary and secondary pericyclic xylem.

In other cases, numerous scattered tracheids appear in the pith below the point of separation of the leaf trace (fig. 9). As the trace leaves the stele, some of these swing out with it and contribute to the vascular supply of the branch. In such cases, there is not the slightest evidence of an internal cambium. Primary centripetal xylem of this kind occurs in the vascular supply of five of the nine branches, while abundant centripetal secondary wood is present in the other four.

It is not necessary to describe in detail the vascular connections of the other branches. Accessory secondary wood usually forms a considerable part of the supply and is present in every case. In six of the nine branches, wings of xylem form between the leaf and the trace and the stele, as in *B. ramosum;* in the other three such formations are entirely lacking. Xylem of pericyclic origin makes up a part of the connection in five cases; in three of these a definite cambium is present. The accessory secondary wood is the only formation which occurs in all the nine cases of this species.

The significance of the wide variation in the vascular supply of the branches will be discussed later.

WOUND REACTIONS

JEFFREY (7) has put forward the view that traumatic reactions are apt to be reversionary in character. BOWER (1) and LANG (9) have used this view in their contention that the pith of the Ophioglossaceae is stelar in character. In the examination of branching specimens of *Botrychium*, especially of *B. obliquum*, some further data upon the nature of the wound reactions of this genus have been secured.

Periderm formation.—The formation of cork at the point of injury has commonly been accepted as a direct response to conditions. In several of the rhizomes of *B. obliquum*, injuries affected all the tissues of the stem. In these cases, periderm is formed by every tissue that is still capable of growth; that is, by cortex, endodermis, pericycle, cambium, and pith. Periderm formation by the pith is shown in fig. 10.

The formation of vascular tissue by the pith.—In three specimens of *B. obliquum*, tracheids scattered through the pith were found in considerable numbers; fig. 9 shows a section through such a stem. In these cases the injury is at some distance above the point of appearance of the pith tracheids.

In both branching specimens of *B. virginianum* and in three of the four of *B. obliquum*, a considerable development of secondary xylem occurs within the pith; figs. 10 and 11 show examples of this. In all cases this development of centripetal secondary xylem occurs below and opposite the point of departure of a leaf trace. The cambium is always directed toward the center of the stele; well developed sieve tubes are present in most cases.

The formation of vascular tissues by the cambium.—In every injured rhizome of *B. virginianum* and *B. obliquum* a renewed activity of the cambium has occurred. Lignification does not always take place in the first elements produced by this renewed activity; this produces a narrow strip of cells of rectangular cross-section just outside the original xylem of the stem. Usually, however, tracheids resembling those of the original growth are produced by this renewed cambial activity (fig. 10); these make up the accessory secondary xylem mentioned above. It is to be noted that no such accessory xylem was found in either of the two species of the section EUBOTRYCHIUM that were examined, but that such formation occurs in *B. Lunaria* as reported by LANG.

The formation of vascular tissues by the pericycle.—In every injured rhizome of *B. virginianum* and *B. obliquum* the pericycle has produced xylem in greater or less amount. As already stated, these masses of pericyclic xylem sometimes contribute to the vascular supply of the branches. When the amount of xylem that occurs is relatively small, only a tangled mass of tracheids is formed; but

frequently a cambium develops and produces tracheids in definite radial rows (fig. 12). The cambium usually occurs on the inner side of the xylem, but in a few cases it is located on the outer side, and sieve tubes occur between it and the endodermis.

The endodermis.—A well marked external endodermis is constantly present in all species of *Botrychium* that were examined. The suberized band on the radial walls is particularly heavy in *B. virginianum* and *B. obliquum.* In uninjured rhizomes of these two species, the external endodermis is continuous except at the points of departure of leaf traces. As a trace swings out from the stele, the endodermis breaks at the side of the trace and closes again rather higher up across the leaf gap; the endodermis on the abaxial side of the trace persists for a short distance only. In injured rhizomes of these two species, the irregular growth of the various stelar tissues has produced distortions and breaks of the endodermal layer (fig. 6). In these specimens, the cells of the endodermis frequently divide by periclinal walls; but there is no evidence that vascular elements are ever formed as a result of this growth. In no case were vascular elements found definitely outside the external endodermis. The only internal endodermis found was that occasionally formed by the folding in of the margins of the external endodermis in cases of greatly disturbed steles (fig. 6, *C*).

Discussion.—The occurrence of scattered tracheids in the pith has been observed in *B. ternatum* by BOWER (1) and in *B. Lunaria* by LANG (9). As stated above, both scattered tracheids and distinct secondary xylem occur frequently in the pith of injured rhizomes of *B. virginianum* and *B. obliquum.* The occurrence of well organized strands of xylem in the pith of a rhizome of *Ophioglossum pendulum* has been reported by the writer (10). In view of these facts, it may be concluded that the pith is definitely stelar in character in *Botrychium* and probably in all three genera of the family.

A slight intrusion of cortical tissue might occur at the point of the break of the endodermis without affecting the pith; such an intrusion would affect only the tissues between the leaf trace and the leaf gap. It may be pointed out that in branching specimens of *B. ramosum*, *B. virginianum*, and *B. obliquum*, xylem is produced

in quantity at this location; hence this tissue is not an intrusion of the cortex, but is stelar in character. It is to be noted that this tissue is opposite a break in the endodermis, but not outside it.

The view is held that there is a morphological distinction between stelar and cortical tissues; that the suberization of the radial walls of a layer of cells is a physiological phenomenon which under the usual conditions of development occurs in the layer of cells next outside the stele; and that this suberization, under the usual conditions, may be considered an indication of the morphological boundary of the stele, but that it is subject to variation with physiological conditions. LANG has concluded that the internal endodermis of *B. Lunaria* is of physiological significance only, and has suggested that its development is associated with the long leaf gaps of the intermediate region of the rhizome. It is to be noted that the apparent internal endodermis in injured specimens of *B. obliquum* (fig. 6, *E*, *F*) occurs between masses of vascular elements and points of injury.

Of the stelar tissues, the tracheids and sieve tubes are incapable of further growth. The remaining tissues are separated by these into three groups: (1) pith, (2) cambium and adjacent parenchyma, and (3) pericycle. The data given above show that all these three tissues may produce vascular elements, either tracheids or sieve tubes. Hence we may conclude that the production of vascular elements by any stelar tissue is limited only by the capacity of the tissue for further growth.

The manner of that further growth of a stelar tissue varies with the species. In *Ophioglossum pendulum*, which has no secondary thickening, the strands of xylem in the pith of an aberrant specimen were primary in origin. In *Helminthostachys*, no secondary xylem is formed under the usual conditions of development; and renewed growth of the stelar tissues, as in the case of branching, produces an irregular secondary thickening of the stele without a definite meristematic layer. On the other hand, *B. virginianum* and *B. obliquum* show very great development of secondary wood under usual conditions. In both these species, renewed growth of the pith, cambium, and pericycle manifested itself in part by the formation of secondary xylem. Hence we may conclude that the

manner of formation of vascular elements by stelar tissues varies with the species, but is relatively constant in any one species under various conditions.

Examination of the various structures shows that they differ mainly in the tissue producing them and in amount of development. That is, these individual variations are differences of position and quantity of vascular elements, and as such their explanation is to be looked for among the physiological factors operating at the time of their development. From this point of view, vascular structures produced as a result of injury may readily show ancestral characters; but such characters are to be considered, not as the repetition of a definite stage of the phylogenetic development of the form, but rather as an indication of the recurrence of certain conditions of development.

It seems well to insist at this point that vascular strands are secondary structures as compared with the tissues which they traverse. The formation of an organ creates a physiological demand to which the vascular strand is a response; and uniformity of the structure which results is only an indication of uniformity of demand and of uniform conditions of development. In this view, the vascular connections of the branches are determined by factors of the same character as those controlling wound reactions.

The vascular supply must be contributed by stelar tissues capable of growth; these tissues are the ones already enumerated, pith, cambium and adjacent parenchyma, and pericycle, together with the parenchyma between the leaf trace and the leaf gap. The manner of formation of the vascular elements of the branch supply is more or less restricted in any species to the particular method of that species. Thus in *B. ramosum,* in which secondary wood formation is relatively slight, no renewed cambial activity occurred in connection with the formation of a branch; while in *B. obliquum,* in which secondary wood formation is very marked under usual conditions, not only does the cambium begin active growth in every case of branching, but similar cambial activity is sometimes set up in both pith and pericycle. The physiological demand likewise varies; in the branch of *B. ramosum* represented in figs. 4 and 8,

two growing points were doubtless established. This produced a physiological demand which, operating under the conditioning factors just described, produced two branch steles.

The conclusion that is reached, therefore, is that the vascular connections of the branches are determined in general by three factors. These factors are (1) the presence of stelar tissues capable of growth within the range of the influence of the developing branch; (2) the nature of the growth which can be induced in those tissues, in particular, whether such growth is cambial or not; (3) the physiological demand produced by the growing branch. The first of these factors will vary with the distance of the bud from the leaf trace as compared with its distance from the stem stele; and, more particularly, with the age of those structures when the branch begins to develop. The second factor may be considered to be relatively constant for any one species but to vary widely with different species. The vascular connection produced is the direct result of the third factor acting upon and limited by the other two; the individual conditions give to this third factor a special value for each particular case; the resultant structures are therefore direct responses to unknown and varying conditions and of physiological interest only. It is therefore concluded that the vascular connections of the branches of *Botrychium* have little or no phylogenetic significance.

THE ORIGIN AND DEVELOPMENT OF THE AXILLARY BUDS

In view of the foregoing conclusion, attention has been turned from the anatomy of the branch connections to the examination of the origin of the axillary buds. Since LANG'S description of these buds in *B. Lunaria* deals only with the mature structures, the further investigation has been directed toward the examination of the origin and development of these buds in *B. obliquum*.

The apical region.—The apical region of *Botrychium* has been described in detail by HOLLE (6), CAMPBELL (3), and BRUCHMANN (2). All agree that growth takes place by means of an apical cell of the form of a triangular pyramid, and that each segment of the apical cell probably gives rise to a leaf. BRUCHMANN states that in *B. Lunaria* leaf formation begins by the appearance of an apical

cell within a segment; and that the entire segment rises abruptly above the plane of the apex of the stem.

In connection with the investigation of the origin of the axillary buds, the apical region of *B. obliquum* has been examined, and the results will be given briefly. As shown by figs. 13 and 14, the apical cell is a triangular pyramid, with three cutting faces. The first division of a segment is by a periclinal wall, as stated for *B. virginianum* by CAMPBELL; the further divisions are irregular.

While the limits of the various segments cannot always be exactly defined, it seems certain that each segment gives rise to a leaf; hence one segment is cut off each year. Fig. 13 represents a transverse section through the apical region of a plant collected early in April; the first segment was cut off during the preceding year and has divided transversely. In the second year, irregular divisions, both longitudinal and transverse, take place with the segment; at the end of the year, the segment consists of 6–15 cells. During the third year, this irregular division continues; but there is little or no extension above the plane of the apex. At the beginning of the fourth year, the segment begins a much more rapid growth and rises abruptly above the plane of the apex, as described by BRUCHMANN; at about the same time, an apical cell is recognizable within the segment, and the further growth is definitely apical. It is evident that while the entire segment takes part in the initial growth, the leaf is formed from only a part of the segment; the remainder of the segment builds up the stem tissues. By the end of the fourth year, the leaf has become a hemispherical mass which has grown forward and upward, and its forward margin extends considerably beyond the apical cell of the stem (fig. 14). Early in the fifth year, the fertile spike makes its appearance. It is first recognized as an apical cell on the forward side of the apical cell of the leaf; by the end of the year, it has produced a well defined knoblike structure. During the sixth year, both the fertile and sterile portions of the leaf develop rapidly. In the summer of the seventh year, this leaf breaks through the base of the enveloping older leaf, and the spores are shed in September. The fertile spike then withers, but the sterile portion of the leaf persists

through the winter, and only dies in the early summer of the eighth year, after the emergence of the next younger leaf.

The bud.—As stated above, the first evidence of the formation of a new leaf is the abrupt rise of the entire segment above the plane of the apex. This growth is relatively slight in amount, and the further development of the young leaf is apical. The cells produced by the apical growth are arranged in such definite rows (fig. 15) that it is easy to distinguish the tissue produced by the apical cell.

The axillary bud can first be distinguished on the adaxial face of the base of a young leaf, after the apical growth of the leaf has proceeded to a considerable extent (fig. 15). At this time, it consists of a plate of meristematic cells, 6–8 in number; there is no evidence of an apical cell. The arrangement of the surrounding cells shows clearly that the bud has not come from the apical cell of the leaf, but has arisen from cells carried up by the elongation of the segment. By the upgrowth of the apical region of the stem, this plate of meristematic cells is thrown into a crevice, which lies between the stem and the adaxial face of the base of the leaf; fig. 14 shows the appearance of a bud a year older than that shown by fig. 15. It consists of a mass of 30–40 cells, lying as a plate at the base of the leaf; one of the cells shown has divided by a periclinal wall, but such a division occurs rarely.

In older leaves, the location of this crevice or slit is readily pointed out by the margin of the stipular sheath of the next younger leaf; this makes the identification of the buds in longitudinal sections of the rhizome particularly easy. Fig. 16 shows the margin of the stipular sheath of the functioning leaf of a rhizome, and the bud formed in the axil of the leaf of the preceding year; fig. 17 shows a bud a year older. They consist of plates of cells of a meristematic nature; these plates are never more than two cells in thickness and are usually 5–8 cells in longitudinal extent. In tangential sections of a rhizome (figs. 18, 19), they are seen to be 6–10 cells in width; the canal by which they communicate with the exterior is a mere slit. There is not the slightest evidence of any apical cell, or other indication of an apex. They agree in all essentials with the buds of *B. Lunaria* as described by LANG.

Discussion.—As stated above, neither axillary nor adventitious buds occur in the rhizomes of either *Ophioglossum vulgatum* or *O. pendulum;* and where branching occurs in these species, it is dichotomous. On the other hand, the buds and branching of *Helminthostachys* resemble those of *Botrychium* in every respect. These facts may be considered further evidence of the close relationship of these two genera; at the same time, they will serve to emphasize the differences between these two genera on the one hand and *Ophioglossum* on the other.

Lang (9) has mentioned the similarity between the vascular connection of the branches of *Botrychium* and of species of the Hymenophyllaceae. For purposes of comparison, the origin of the axillary buds of a species of *Trichomanes* from Samoa has been examined. The leaf has an apical cell from the beginning and develops to a considerable extent before the branch appears. The formation of the branch is initiated by the appearance of an apical cell. The exact method of formation of this apical cell of the branch was not determined; but it is evident that it arises late and from the growing point of the leaf. The axillary branches of *Trichomanes*, therefore, are foliar in origin. In *Botrychium*, as shown above, the axillary bud is in no way related to the apical cell of the leaf; it arises directly from a portion of a segment of the apical cell of the rhizome; and its position on the base of the leaf is incidental and does not indicate a foliar origin. The similarity between the branching of *Botrychium* and *Trichomanes*, therefore, is not close.

The mature axillary buds of *Botrychium* are of the simplest possible form, an undifferentiated layer of meristematic cells; and it is to be noted that at no time in their development is there any differentiation. This may be accepted as evidence of reduction produced in connection with dormancy; and we may conclude that *Botrychium* and *Helminthostachys* have been derived from a form which branched freely in a monopodial fashion. This is in full agreement with other evidence which points to a relationship of the Ophioglossaceae to the primitive forms of Filicales, especially the Zygopterideae.

Summary

1. Branching of the rhizome of *Ophioglossum vulgatum* and *O. pendulum* is dichotomous; there are no axillary or adventitious buds on the rhizome.

2. Axillary buds are regularly present in five species of *Botrychium.*

3. The vascular connections of the branches in *Botrychium* vary widely with the species and with the individual specimen. It is concluded that the details of the vascular supply of the branch are controlled by the conditions of development and are therefore of little or no phylogenetic importance.

4. In wounded rhizomes of *B. obliquum*, renewed activity of the cambium produces considerable masses of accessory xylem; the pith frequently develops sieve tubes and a cambium which produces secondary xylem in quantity; the pericycle often produces sieve tubes and secondary xylem. It is concluded that in this species any stelar tissues capable of growth may produce vascular elements under the influence of an injury.

5. The axillary bud of *B. obliquum* arises as a plate of meristematic cells on the adaxial face of the base of the very young leaf; it develops without differentiation into a plate of meristematic tissue one or two cells in thickness and 50–60 cells in area, which is buried by overgrowth of surrounding tissue.

6. The data secured is in agreement with the evidence pointing to a relationship of the Ophioglossaceae to the primitive ferns, especially the Zygopterideae.

Syracuse University
Syracuse N.Y.

The writer wishes to acknowledge the kindness of Professor John M. Coulter in placing the facilities of the Hull Botanical Laboratory at his disposal; and to express his thanks to Dr. W. J.G. Land for material of *Trichomanes*.

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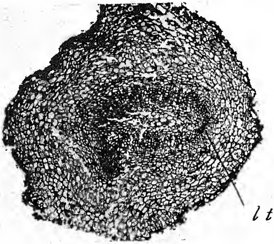
EXPLANATION OF PLATES XX AND XXI

(Figs. 1–6 are in the text)

FIG. 7.—Transverse section of a rhizome of *Botrychium ramosum* at the base of a branch: *lt,* leaf trace; ×20.

FIG. 8.—Transverse section of the branch whose origin is represented by figs. 4 and 7, showing the two branch steles: *a,* larger stele of the branch; *b,* smaller stele of the branch; *r,* trace of root which connects with the smaller branch stele; ×20.



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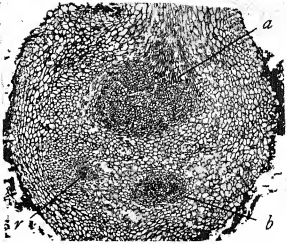

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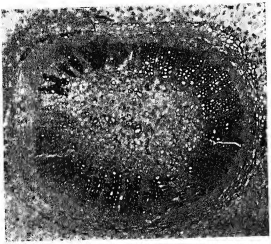

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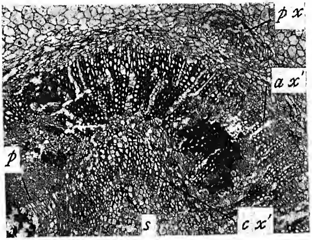

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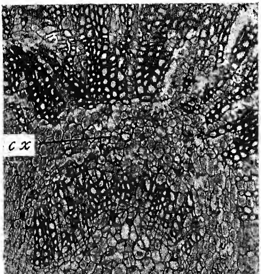

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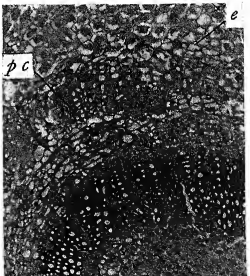

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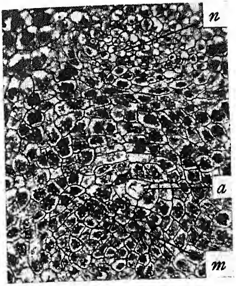
PETRY on OPHIOGLOSSACEAE



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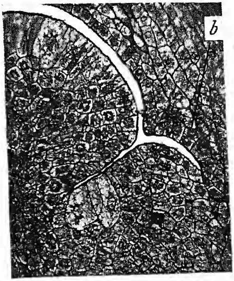

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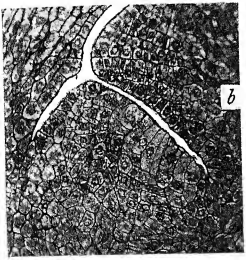

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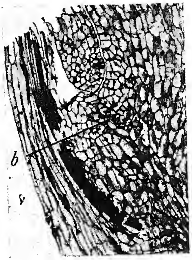

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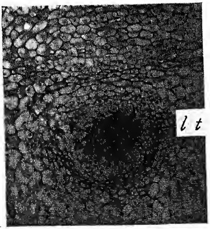

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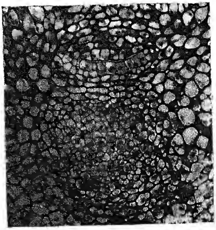

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PETRY on OPHIOGLOSSACEAE

FIG. 9.—Transverse section of a stele of *B. obliquum*, showing tracheids scattered through the pith; ×20.

FIG. 10.—Transverse section of a portion of the stele of an injured rhizome of *B. obliquum*, showing development of new vascular elements in connection with the formation of a branch: *cx'*, centripetal secondary xylem; *ax'*, accessory secondary xylem; *px*, pericyclic xylem; *s*, sieve tubes; *p*, periderm; ×27.

FIG. 11.—Transverse section of a portion of the stele shown in fig. 10, at a lower level, and showing detail of the vascular elements in the pith; *cx*, centripetal primary xylem; ×48.

FIG. 12.—Transverse section of a portion of a stele of *B. virginianum*, showing secondary xylem in the pericycle: *pc*, pericyclic cambium; *e*, endodermis; ×48.

FIG. 13.—Transverse section through the apical region of a rhizome of *B. obliquum:* *a*, apical cell; *m*, procambium of the youngest leaf; *n*, procambium of the next older leaf; ×122.

FIG. 14.—Longitudinal section through the apical region of a rhizome of *B. obliquum:* *b*, axillary bud of second leaf; ×122.

FIG. 15.—Longitudinal section through the apical region of the rhizome of *B. obliquum:* *b*, axillary bud of the youngest leaf; ×122.

FIG. 16.—Longitudinal section through axillary bud of *B. obliquum;* ×48.

FIG. 17.—Longitudinal section of mature bud of *B. obliquum;* ×48.

FIG. 18.—Transverse section of axillary bud near its base, as seen in a tangential section of the rhizome: *lt*, trace of the subtending leaf; ×48.

FIG. 19.—Transverse section of bud near the surface of the rhizome; ×48.

VARIATIONS IN RESPIRATORY ACTIVITY IN RELATION TO SUNLIGHT

H. A. Spoehr

(WITH TEN FIGURES)

Although the importance of light as a physiological stimulus and as a climatological factor has long been recognized, the complexity and multiplicity of biological light reactions has but recently been realized. As a working hypothesis, I have endeavored to analyze the light reactions by dividing them into two classes: (1) those reactions which are brought about by the light directly inducing chemical or physical changes of certain physiologically important substances *within the organism;* and (2) those reactions which are caused by the light affecting the *environment of the organism.* In the first class fall such reactions as the reduction of the acidity of the plant juices[1] with the consequent effects on growth, the inversion of disaccharides, and a number of other purely photochemical reactions. This paper is a prefatory announcement of a reaction which I believe to belong to the second class. It is my hope, by means of such an analysis, eventually to be able to interpret climatological light reactions upon a sound physiological basis.

As one of the most simply measurable plant activities, I have chosen respiration, as indicated by the evolution of carbon dioxide. The great complexity of the chemistry of this process in no wise affects the results for the present purpose.

The effect of light upon the respiratory activity of living things in general has received considerable attention. Unfortunately, however, in most of these investigations the source and nature of the light used were not considered, and hence we have a mass of uncoordinated and often contradictory results. In 1855 Moleschott[2]

[1] Richards, H. M., Reports in Yearbook Carnegie Inst. Wash. 1913 and 1914. Spoehr, H. A., Photochemische Vorgänge bei der diurnalen Entsäurung der Succulenten. Biochem. Zeitschr. 57:95–111. 1913.

[2] Moleschott, Jac., Die internationale Sanitäts Konferenz in Rom. Wiener Med. Wochenschr. 1855. nos. 36–38.

noticed the increased carbon dioxide production of the frog under the influence of light, and later the same fact was observed in man by Pettenkofer and Voit[3] and others. However, in the higher animals the results become complicated by the action of the light on the nervous system.[4] The experiments on plants have been made mostly with fungi, but on account of the confusing influences of temperature, nutrient media, and sources of light, no definite conclusions can be drawn from these investigations.[5]

Recently, Meyer and Deleano[6] have found that the carbon dioxide production of leaves at practically constant temperature and in the dark is decidedly higher during the daytime than at night. These workers consider the cause of this variation as lying within the organism. They say:

> Nachdem wir wissen, dass die Aufpraegung einer intermittiernden chronometrischen Bewegungsstruktur, welche zur periodischen Erhoehung der Kohlensaeureproduktion fuehrt, moeglich ist, duerfen wir wohl die Hypothese aufstellen, dass die regelmaessigen Schwankungen der Kohlensaeureproduktion an Stunden des Volltages bei normalen Laubblaettern nur durch den waehrend des Volltages stattfindenden Wechsel der Assimilationsintensitaet und wahrscheinlich erst waehrend ihres individuellen Lebens des Laubblattes hervorgerufen ist.

In the following experiments (mostly with wheat seedlings), the carbon dioxide production in the dark at constant conditions of temperature and humidity was measured by drawing air from out-of-doors over the plants, and then through a standard barium hydroxide solution. It was found that the rate at which carbon dioxide was produced during the hours of daytime was regularly higher than that produced during the night. It is evident that under these experimental conditions the only variable external

[3] Pettenkofer, M., and Voit, C., Über Kohlensäureausscheidung und Sauerstoffaufnahme während des Wachens und Schlafens beim gesunden u. kranken Menschen. Sitzber. Akad. Wiss. München 2:236. 1866.

[4] Neuberg, C., Beziehungen des Lebens zum Licht. Berlin. 1913 (p. 7).

[5] Kolwitz, R., Über den Einfluss des Lichtes auf die Athmung der niederen Pilze. Jahrb. Wiss. Bot. 33:128. 1899.

Maximow, N. A. *Ibid.* Bot. Centralbl. 90:94. 1902.

[6] Meyer, A., and Deleano, N. T., Die periodischen Tag- und Nachtschwankungen der Atmungsgroesse im Dunkeln befindlicher Laubblätter und deren vermutliche Bezeihung zur Kohlensäureassimilation. Zeitschr. Bot. 3:658–701. 1911; 5:209–320. 1913.

condition to which the plants were exposed was the air of the daytime on the one hand, and that of the night on the other. Are there then any differences in the atmospheric air during day and night which might account for this remarkable variation in the respiratory activity? The first thing to suggest itself is the possible influence of the sunlight on the atmosphere.

The intensity of the violet and ultra-violet rays of the sunlight, as measured at the Desert Laboratory during 18 months, compared with the values generally given for atmospheric ionization, showed a remarkable similarity. DEMBER[7] and others[8] have reported the same fact from observations in other localities. Physicists are not agreed as to the exact relation between sunlight and atmospheric ionization,[9] nor is a discussion of this subject necessary for the present purpose. Suffice it to note that according to the observations of ELSTER and GEITEL, A. GOCKEL, VON SCHWEIDLER, and others, atmospheric ionization exhibits a main maximum about noon, a secondary maximum a little before sunrise, with minima after sunset and before sunrise. These values are subject to certain variations, since the ionization is affected by other meteorological factors, as is mentioned below.

In general, then, the highest respiratory activity takes place during the period of increased ionization. If the respiratory activity and atmospheric ionization are in any way related, the artifical deionization of the air drawn over the plants should have a marked effect on their respiratory activity under the present experimental conditions.

[7] DEMBER, H., Über die ionisierende Wirkung des ultravioletten Sonnenlichts. Physik. Zeitschr. 13:207–212. 1912.

[8] KAEHLER, K., Luftelektrizität. Leipzig. 1913 (p. 69).

[9] LENARD, P., and RAMSAUER, C., Über die Wirkung Ultravioletten Lichtes auf Gase unter besonderer Berücksichtigung der Vorgänge in der Erdatmosphaere. Metrol. Zeitzchr. 29:150. 1912.

LENARD, P., Über die Wirkung des Ultravioletten Lichtes auf Gasförmige Körper. Ann. Phys. 1:486. 1900; 3:298. 1900.

THOMSON, J. J., Conduction of electricity through gases. Cambridge. 1913 (p. 254).

ELSTER, J., and GEITEL, H., Die Existenz elektrischer Ionen in der Atmosphäre. Jour. Terr. Magnetism and Atmos. Elect. 4:213. 1899.

STARK, J., Die Elektrizität in Gasen. Leipzig. 1902.

Methods and apparatus

In fig. 1 the apparatus used in this investigation is schematically shown; all of the tubes are of glass with heavy rubber connections. In order to avoid all possible contamination of the air, this was drawn from out-of-doors on the north side of the laboratory building, through a glass tube, and entered the apparatus at E_1. The air was not drawn through any liquid in order to avoid submitting the plants to changes of pressure, and so as not to affect the electrical conditions of the atmosphere. The large bottle (*B*) contained a 50 per cent aqueous solution of potassium hydroxide; the glass tube was placed so that the air passed immediately over the solution. This bottle was omitted in the experiments of short duration. In order to further remove the carbon dioxide, the air was passed through the bulb tubes (*C*); the lower portion of the bulbs were filled with 50 per cent potassium hydroxide solution. The tubes were so arranged that they could be shaken from time to time. *S* represents a glass tube, one inch in diameter, containing coarse soda lime (4 mesh). The air then passed into a Frease electrical thermostat (*T*). This was kept at 29° in all of the experiments given below. It was, of course, of great importance that the air coming in contact with the plants should also attain the same constant temperature. In order to do this, the air was first passed into a 500 cc. Erlenmeyer flask (*F*), then through 45 feet of thin-walled glass tubing (*G*), and finally into the flask *R* containing the plants. As there was always a small amount of water carried out with the air current, this was condensed and trapped at *W*. The rate at which the air was allowed to pass through the entire apparatus was controlled by means of the glass stopcock *A*. It was found that variations in the rate of air from 0.5 to 8 liters per hour caused no difference in the rate of carbon dioxide evolution. P_1 and P_2 represent three-way glass stopcocks, by means of which the course of the air could be changed from one to the other of the Meyer's tubes (*M*) without interrupting the experiment. The Meyer's tubes were found far more satisfactory for the absorption of carbon dioxide than Pettenkofer tubes or any of the modifications of the latter. Especially was this true in the experiments

running 12–15 hours. The absorption tubes were inserted by making the connection at *L* with heavy rubber tubing; 125 cc.

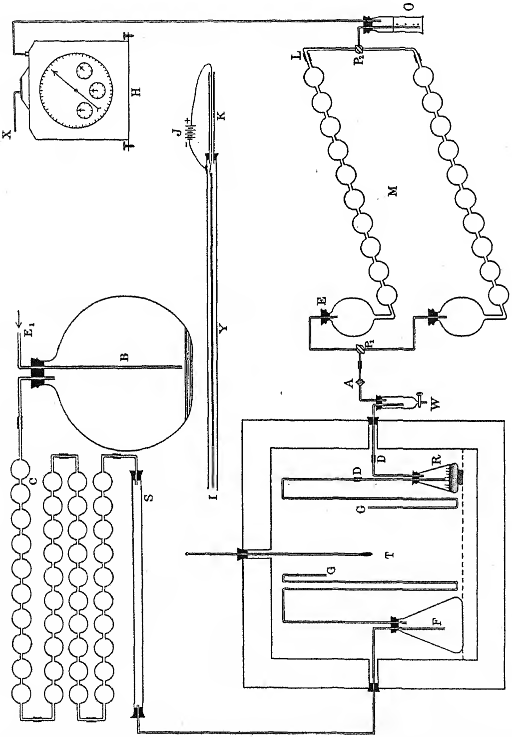

FIG. 1.

of the approximately 0.1 normal barium hydroxide solution was then run into the large bulb, and the connection made at *E* with a

rubber stopper. When the amount of carbon dioxide is large, it is, of course, advisable to use 0.5 normal solution. The tube was then ready for use by turning P_1 and P_2. The error caused by the amount of carbon dioxide of the air in the large bulb was found to be negligible for these experiments. While one tube was in use, the other could be charged. After having drawn the air through one tube for the required length of time, the other was inserted into the system, and the barium hydroxide solution from the first plus the precipitated barium carbonate poured into a narrow bottle, well stoppered, and the barium carbonate allowed to settle for 24 hours; 25 cc. of the clear supernatant solution was then titrated with 0.1 normal hydrochloric acid. From the difference between the amount of acid required for the used barium hydroxide solution and the original solution, the amount of carbon dioxide evolved can be very simply calculated. In the figure, *O* is a small wash bottle containing barium hydroxide solution, to detect any escaping carbon dioxide beyond the Meyer's tubes; this was never found to take place. The rate of air flow was measured by means of the gas meter *H*. At *X* connection was made with a Palladin pressure regulator, and this was attached to an electrically driven suction pump.

The nature of the plant material used for these experiments was found to be of greatest importance. Briefly, it is necessary that the organisms be actively respiring, and that the gaseous exchange with the atmosphere be not too difficult. For instance, potato tubers evolve carbon dioxide so slowly that even with a large quantity the differences between day and night are exceedingly slight. There must also be a sufficient supply of carbohydrate food material, for it was found that as soon as the carbohydrates were exhausted, the nature of the carbon dioxide evolution changed greatly.[10] The difficulty with wheat seedlings is, of course, that the relatively rapid rise and fall of the rate of carbon dioxide evolution makes a comparison between day and night

[10] Compare Deleano, N. T., Untersuchungen über den Atmungsstoffwechsel abgeschnittener Blätter. Jahrb. Wiss. Bot. 51:541–593. 1912. He found that only after the exhaustion of the carbohydrates was there any evidence of protoplasmic disturbance.

rather difficult. I believe, however, that for the present purpose these experiments will serve as the best illustration.

Before starting an experiment, the wheat grains were always sterilized: *R*, an Erlenmeyer flask (of 100 cc. for the experiments with 70 seedlings, 1000 cc. when 200 seedlings were used) was provided with a rubber stopper and glass tubing which could be detached at *D*. The bottom of the flask was covered with about an inch of glass wool, and the flask and tubing were then sterilized in the autoclave. The wheat grains were placed in a small cheesecloth sack, immersed for three minutes in a concentrated aqueous solution of chloroform, and shaken to free the seeds from adhering air bubbles. As much as possible of the chloroform solution was removed, and the contents quickly emptied into the sterile flask. Dry, carefully filtered air was then drawn through the flask for at least 24 hours until the seeds were perfectly air dry, so that no trace of chloroform could remain in the flask. Sterile water was then added through one of the tubes; these were then connected at *D*. Material treated in this way very rarely developed any growth of fungus during the course of the experiment, nor did it show any differences in growth as compared with untreated seeds. It is highly improbable, therefore, that the seed coats were penetrated by the chloroform solution. It was found that the wheat could also be sterilized by means of ultra-violet light. This is an exceedingly convenient method, but as it was found that the subsequent growth of the seedlings was somewhat affected, the method was not used for these experiments.

The deionizing apparatus used consisted of a brass tube (*Y*) five feet long and one inch in diameter. Into this was concentrically placed an iron rod, the same length and one-quarter inch in diameter, and held by fiber supports. The tube and rod were attached to the opposite poles of a series of batteries of 50 volts and 800 amperes. To one end of the brass tube was connected a glass tube containing cotton, to act as a filter. The deionizing apparatus was connected at *I* with *E*.

Blank experiments were, of course, always run in order to test the apparatus. In experiments 1, 2, 4, and 5 it will be noted that the night periods are longer than the day periods. It is conceivable

that drawing air through the barium hydroxide solution would evaporate some water, thus make the solution more concentrated, and hence give lower values for the longer periods. The following blank experiment eliminates this possibility.

25cc. original $Ba(OH)_2$ solution	= 27.19cc. 0.1 NHCl	
25cc. after 3.5 hours	= 27.05cc.	" "
25cc. after 16 hours	= 26.99cc.	" "

That differences in atmospheric pressure might account for the daily variation in carbon dioxide evolution also suggested itself. The exceedingly slight variations of pressure at Tucson bear no possible similarity to the values of respiration.

Experimental results

The following is a brief account of a few typical experiments carried out during the winter of 1913–1914. The first column in the tables gives the number of the carbon dioxide determination; the second, the time of day during which it ran; the third, the number of hours; the fourth, the rate per hour at which the air was drawn over the plants in liters; the fifth, the rate of carbon dioxide evolved per hour, expressed in milligrams. In the sixth column the weather is roughly indicated: *A* stands for a perfectly cloudless day; *B*, a few scattered cumulus clouds; *C*, more clouds than *B*, usually thin cirrus; *O*, overcast; and *R*, rain.

The curves are plotted with the rate of carbon dioxide evolution per hour in mg. on the ordinates, the successive determinations on the abscissas; the broken lines indicate the day rates, the solid lines the night rates.

(1) The first experiments (p. 374) were made with small onion bulbs (Australian brown) 0.75–1 inch in diameter. In order to insure ease of gaseous exchange in the onions, the dry outer layers were removed. Previous experiments showed that this operation resulted in but a very slight traumatic effect. After 24 hours, 35 of these small onions were placed in flask *R*. To prevent drying out, the air was drawn over water contained in a bottle inserted between the tubes *G* and the flask *R*. The experiment ran from January 21 to February 2. The results are given in table I and fig. 2. Here it can be seen that the differences in carbon dioxide

TABLE I

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	10:30 A.M.– 5:30 P.M.	7.00	6.86	4.47	A–B
2	5:30 P.M.– 8:30 A.M.	15.00	6.14	3.45	A
3	8:30 A.M.– 5:30 P.M.	9.00	5.78	3.21	C
4	5:30 P.M.– 8:30 A.M.	15.00	5.93	2.57	O
5	8:30 A.M.– 5:00 P.M.	8.50	5.65	2.42	O
6	5:00 P.M.–10:00 A.M.	17.00	5.76	2.13	C
7	10:00 A.M.– 3:15 P.M.	5.25	5.52	2.20	O
8	3:15 P.M.–10:15 A.M.	19.00	5.42	1.98	O
9	10:15 A.M.– 6:15 P.M.	8.00	5.12	1.89	C
10	6:15 P.M.– 9:00 A.M.	14.75	4.95	1.76	O
11	9:00 A.M.– 4:45 P.M.	7.75	4.91	2.11	O
12	4:45 P.M.– 8:30 A.M.	15.75	4.95	1.96	O
13	8:30 A.M.– 5:00 P.M.	8.50	4.82	1.81	O
14	5:00 P.M.– 8:15 A.M.	15.25	4.79	1.67	R
15	8:15 A.M.– 5:15 P.M.	9.00	4.89	1.61	C–A
16	5:15 P.M.– 8:45 A.M.	15.25	4.65	1.58	A
17	8:45 A.M.– 5:30 P.M.	8.75	4.46	1.56	A
18	5:30 P.M.– 8:45 A.M.	15.25	4.39	1.50	A
19	8:45 A.M.– 5:30 P.M.	8.75	4.23	1.67	A
20	5:30 P.M.– 8:30 A.M.	15.00	3.93	1.63	A
21	8:30 A.M.– 5:30 P.M.	9.00	6.22	1.56	A
22	5:30 P.M.– 9:00 A.M.	15.50	6.71	1.45	B
23	9:00 A.M.– 6:00 P.M.	9.00	7.00	1.43	B
24	6:00 P.M.–10:15 A.M.	16.25	6.89	1.56	A

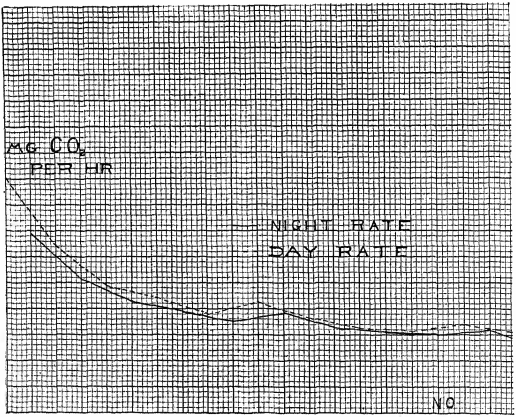

FIG. 2.

production between day and night grow less as the food material is used up, and as the gaseous exchange becomes more difficult, because the shriveled outer layer forms a protecting coat. The amount of carbon dioxide evolved from onions in which the outer layer is dry and contains little food material is very small. $\frac{\text{Day rate}}{\text{Night rate}}=1.15.$

(2) In the next experiment (p. 376) 75 wheat seedlings were used. This was started after the seedlings were 24 hours old, and ran from February 11 to 24. The results are given in table II and fig. 3. $\frac{\text{Day rate}}{\text{Night rate}}=1.042.$

(3) In the experiment tabulated on p. 377, 70 wheat seedlings were used, which ran from April 29 to May 9. Several of the seeds were infected with fungus, wherefore the experiment was not run longer. The results are given in table III and fig. 4. $\frac{\text{Day rate}}{\text{Night rate}}=1.091.$

(4) The deionizing apparatus was used in the next experiment (p. 378), and 70 wheat seedlings were used. The experiment ran from April 14 to April 25. Unfortunately, however, on the night of April 17–18 the pump was out of order, and the determination for that period is not reliable. The results are given in table IV and fig. 5.

(5) Here also (p. 379) the deionizing apparatus was used, with 70 wheat seedlings in the flask. The experiment ran from March 13 to 20. The results are given in table V and fig. 6. $\frac{\text{Day rate}}{\text{Night rate}}=1.010.$

(6) The deionizing apparatus was used here (p. 380), with 70 wheat seedlings. The experiment ran from May 11 to 22. The results are given in table VI and fig. 7. $\frac{\text{Day rate}}{\text{Night rate}}=1.015.$

(7) In this experiment (p. 381) the carbon dioxide was determined for three hour periods, and 200 wheat seedlings were used. The experiment ran from May 25 to 27, and the deionizing apparatus was not used. The results are given in table VII and fig. 8. At this time of the year the night time must be considered as falling within

TABLE II

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	5:45 P.M.– 8:45 A.M.	15.00	2.27	2.68	A
2	8:45 A.M.– 5:30 P.M.	8.75	3.09	4.54	A
3	5:30 P.M.– 8:15 A.M.	14.75	4.41	5.59	B
4	8:15 A.M.– 6:00 P.M.	9.75	4.21	6.98	B
5	6:00 P.M.– 9:00 A.M.	15.00	4.40	8.10	B
6	9:00 A.M.– 6:00 P.M.	9.00	4.22	9.20	B–C
7	6:00 P.M.– 9:00 A.M.	15.00	4.13	9.28	C
8	9:00 A.M.– 6:00 P.M.	9.00	3.89	9.50	B–C
9	6:00 P.M.– 9:00 A.M.	15.00	4.00	8.84	O
10	9:00 A.M.– 7:00 P.M.	10.00	2.80	8.67	C
11	7:00 P.M.– 9:30 A.M.	14.50	2.83	8.12	R
12	9:30 A.M.– 7:30 P.M.	10.00	4.90	8.30	R
13	7:30 P.M.– 9:30 A.M.	14.00	5.21	8.16	R
14	9:30 A.M.– 7:30 P.M.	10.00	3.90	8.45	O
15	7:30 P.M.– 9:30 A.M.	14.00	4.00	8.20	R
16	9:30 A.M.– 8:30 P.M.	11.00	3.91	7.64	B
17	8:30 P.M.–10:00 A.M.	13.50	4.08	6.93	B
18	10:00 A.M.– 8:00 P.M.	10.00	3.80	6.60	B
19	8:00 P.M.–10:00 A.M.	14.00	4.14	6.00	B
20	10:00 A.M.– 7:15 P.M.	9.25	4.22	5.70	C
21	7:15 P.M.– 9:15 A.M.	14.00	4.07	5.10	R
22	9:15 A.M.– 6:15 P.M.	9.00	4.00	4.78	B
23	6:15 P.M.– 9:15 A.M.	15.00	4.07	4.20	B
24	9:15 A.M.– 6:00 P.M.	8.75	4.00	3.88	O

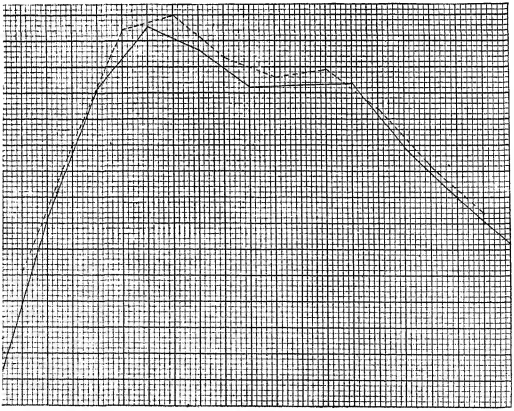

FIG. 3.

TABLE III

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	8:30 P.M.– 8:15 A.M.	11.75	0.76	0.36	B–C
2	8:15 A.M.– 7:45 P.M.	11.50	0.69	0.90	B
3	7:45 P.M.– 8:15 A.M.	12.50	0.96	1.19	B
4	8:15 A.M.– 7:45 P.M.	11.50	1.74	1.76	B–C
5	7:45 P.M.– 8:30 A.M.	12.75	1.88	2.13	B
6	8:30 A.M.– 8:15 P.M.	11.75	1.88	2.62	B–C
7	8:15 P.M.–11:00 A.M.	14.75	2.03	2.97	B
8	11:00 A.M.– 8:45 P.M.	9.75	1.95	3.26	B
9	8:45 P.M.– 8:15 A.M.	11.50	1.83	3.34	A
10	8:15 A.M.– 8:15 P.M.	12.00	1.67	3.46	B
11	8:15 P.M.– 8:30 A.M.	12.25	1.63	3.77	B
12	8:30 A.M.– 8:00 P.M.	11.50	1.83	4.01	O
13	8:00 P.M.– 7:45 A.M.	11.75	1.96	4.16	B
14	7:45 A.M.– 8:00 P.M.	12.25	1.55	4.27	B–A
15	8:00 P.M.– 8:30 A.M.	12.50	1.76	4.40	A
16	8:30 A.M.– 8:00 P.M.	11.50	1.83	4.69	B
17	8:00 P.M.– 8:45 A.M.	12.75	2.12	4.86	B–C
18	8:45 A.M.– 8:00 P.M.	11.75	2.13	5.04	O
19	8:00 P.M.– 8:15 A.M.	12.25	2.28	4.95	C
20	8:15 A.M.– 5:00 P.M.	8.75	2.17	5.06	B

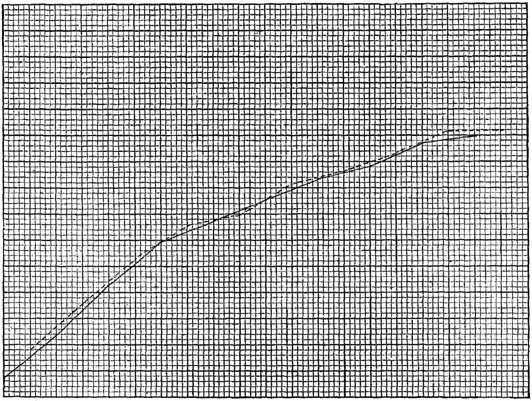

FIG. 4.

TABLE IV

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	4:00 P.M.– 8:30 A.M.	16.50	1.70	0.35	A
2	8:30 A.M.– 5:30 P.M.	9.00	1.33	1.34	A
3	5:30 P.M.– 8:45 A.M.	15.25	1.44	2.73	A
4	8:45 A.M.– 5:30 P.M.	8.75	1.37	3.74	A
5	5:30 P.M.– 9:00 A.M.	15.50	1.37	4.64	A
6	9:00 A.M.– 5:15 P.M.	8.25	1.45	5.80	A
7	5:15 P.M.– 9:30 A.M.	16.25	1.05	....	A
8	9:30 A.M.– 5:45 P.M.	8.25	1.21	5.99	A
9	5:45 P.M.– 8:00 A.M.	14.25	1.33	7.41	A
10	8:00 A.M.– 6:15 P.M.	10.25	1.17	6.91	A
11	6:15 P.M.– 8:30 A.M.	14.25	1.26	6.60	A
12	8:30 A.M.– 5:30 P.M.	9.00	1.12	6.25	B
13	5:30 P.M.– 8:45 A.M.	15.25	1.18	6.14	O
14	8:45 A.M.– 6:30 P.M.	9.75	1.13	6.18	O
15	6:30 P.M.– 8:30 A.M.	14.00	1.28	6.12	B
16	8:30 A.M.– 6:15 P.M.	9.75	1.13	6.35	B
17	6:15 P.M.– 8:30 A.M.	14.25	1.19	6.23	B–C
18	8:30 A.M.– 5:00 P.M.	8.50	1.06	6.18	C
19	5:00 P.M.– 8:15 A.M.	15.25	1.11	5.74	B–A
20	8:15 A.M.– 5:15 P.M.	9.00	1.00	4.82	A
21	5:15 P.M.– 8:00 A.M.	14.75	0.68	4.67	A
22	8:00 A.M.– 2:45 P.M.	6.75	0.59	4.55	A

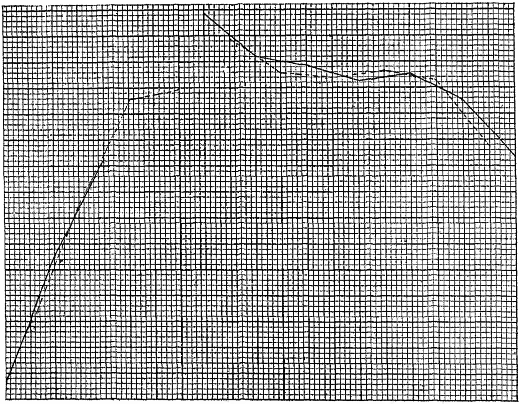

FIG. 5.

TABLE V

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	5:00 P.M.–8:30 A.M.	15.50	3.42	8.14	A
2	8:30 A.M.–6:00 P.M.	9.50	3.90	10.95	A
3	6:00 P.M.–8:30 A.M.	14.50	3.93	12.65	O
4	8:30 A.M.–6:15 P.M.	9.75	4.00	14.81	O
5	6:15 P.M.–8:30 A.M.	14.25	3.93	15.88	A
6	8:30 A.M.–7:30 P.M.	11.00	4.19	16.01	B
7	7:30 P.M.–8:30 A.M.	13.00	4.08	16.45	A
8	8:30 A.M.–5:30 P.M.	9.00	3.11	16.07	B–C
9	5:30 P.M.–8:30 A.M.	15.00	2.93	15.55	O
10	8:30 A.M.–7:30 P.M.	11.00	2.82	15.27	B–C
11	7:30 P.M.–9:00 A.M.	13.50	2.89	14.28	O
12	9:00 A.M.–5:30 P.M.	8.50	3.76	13.14	R
13	5:30 P.M.–8:30 A.M.	15.00	3.87	12.10	B
14	8:30 A.M.–5:30 P.M.	9.00	3.77	11.83	O

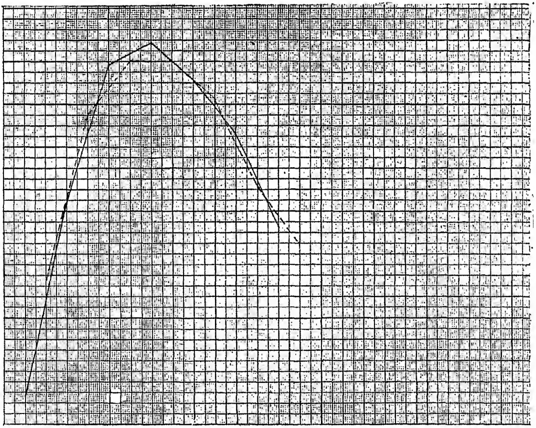

FIG. 6.

TABLE VI

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	9:15 P.M.–8:45 A.M.	11.50	1.65	0.34	C
2	8:45 A.M.–7:00 P.M.	10.25	1.94	0.96	A
3	7:00 P.M.–8:45 A.M.	13.75	1.74	2.03	B
4	8:45 A.M.–8:00 P.M.	11.25	1.91	3.01	O
5	8:00 P.M.–8:15 A.M.	12.25	1.96	3.79	O
6	8:15 A.M.–8:00 P.M.	11.75	1.96	4.56	B
7	8:00 P.M.–8:00 A.M.	12.00	1.83	5.09	A
8	8:00 A.M.–8:45 P.M.	12.75	1.80	5.92	B
9	8:45 P.M.–8:00 A.M.	11.25	1.88	6.20	A
10	8:00 A.M.–8:00 P.M.	12.00	1.93	6.14	O
11	8:00 P.M.–8:00 A.M.	12.00	1.83	6.23	B–A
12	8:00 A.M.–8:00 P.M.	12.00	1.83	6.27	B–A
13	8:00 P.M.–8:15 A.M.	12.25	2.04	5.86	A
14	8:15 A.M.–8:00 P.M.	11.75	1.96	6.11	B
15	8:00 P.M.–8:00 A.M.	12.00	1.93	5.96	A
16	8:00 A.M.–8:45 P.M.	12.75	1.96	5.46	A
17	8:45 P.M.–8:15 A.M.	11.50	2.00	6.65	A
18	8:15 A.M.–8:45 P.M.	12.50	2.18	6.89	B
19	8:45 P.M.–8:30 A.M.	11.75	2.05	7.05	A
20	8:30 A.M.–8:45 P.M.	12.25	2.04	7.31	A
21	8:45 P.M.–8:00 A.M.	11.25	3.70	6.98	B
22	8:00 A.M.–4:15 P.M.	8.25	2.01	5.37	C

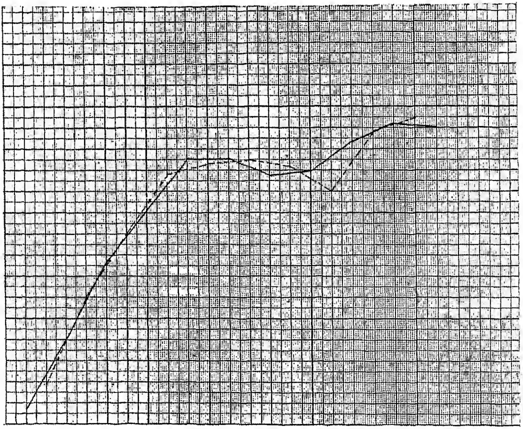

FIG. 7.

TABLE VII

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	12:00 N. – 3:00 A.M.	3	2.00	10.93	A
2	3:00 A.M.– 6:00 A.M.	3	2.33	10.93	A–B
3	6:00 A.M.– 9:00 A.M.	3	2.33	11.88	B
4	9:00 A.M.–12:00 M.	3	2.33	12.47	B
5	12:00 M. – 3:00 P.M.	3	2.00	12.16	B
6	3:00 P.M.– 6:00 P.M.	3	2.33	12.32	B
7	6:00 P.M.– 9:00 P.M.	3	2.33	13.52	B
8	9:00 P.M.–12:00 N.	3	2.33	13.31	A
9	12:00 N. – 3:00 A.M.	3	2.33	14.41	A
10	3:00 A.M.– 6:00 A.M.	3	2.33	14.20	A
11	6:00 A.M.– 9:00 A.M.	3	2.66	15.62	A
12	9:00 A.M.–12:00 M.	3	2.00	15.39	B
13	12:00 M. – 3:00 P.M.	3	2.33	16.01	A
14	3:00 P.M.– 6:00 P.M.	3	2.00	15.91	B
15	6:00 P.M.– 9:00 P.M.	3	2.33	17.11	A
16	9:00 P.M.–12:00 N.	3	2.33	17.22	A
17	12:00 N. – 3:00 A.M.	3	2.00	17.77	A
18	3:00 A.M.– 6:00 A.M.	3	2.00	17.16	A
19	6:00 A.M.– 9:00 A.M.	3	1.67	17.87	A
20	9:00 A.M.–12:00 M.	3	1.67	18.62	A
21	12:00 M. – 3:00 P.M.	3	1.67	19.10	A
22	3:00 P.M.– 6:00 P.M.	3	2.00	18.81	A
23	6:00 P.M.– 9:00 P.M.	3	1.33	19.36	A
24	9:00 P.M.–12:00 N.	3	1.67	18.74	A

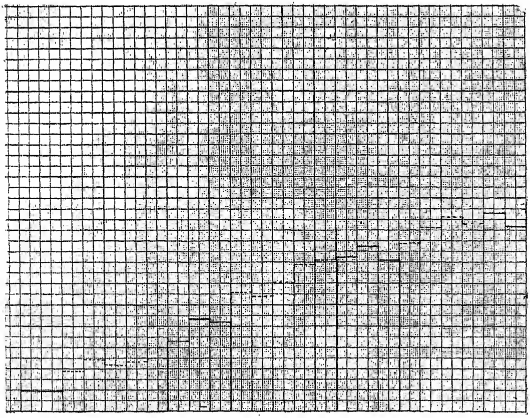

FIG. 8.

TABLE VIII

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	6:00 P.M.– 9:00 P.M.	3	2.67	10.12	B
2	9:00 P.M.–12:00 N.	3	2.00	10.40	B
3	12:00 N. – 3:00 A.M.	3	2.00	11.72	B
4	3:00 A.M.– 6:00 A.M.	3	2.33	12.61	B
5	6:00 A.M.– 9:00 A.M.	3	2.00	13.71	C
6	9:00 A.M.–12:00 M.	3	2.00	14.96	C
7	12:00 M. – 3:00 P.M.	3	2.00	15.72	C
8	3:00 P.M.– 6:00 P.M.	3	2.00	17.00	C
9	6:00 P.M.– 9:00 P.M.	3	2.33	17.30	C
10	9:00 P.M.–12:00 N.	3	2.33	17.60	C
11	12:00 N. – 3:00 A.M.	3	2.33	18.42	B
12	3:00 A.M.– 6:00 A.M.	3	2.33	19.36	B–C
13	6:00 A.M.– 9:00 A.M.	3	2.33	19.68	C
14	9:00 A.M.–12:00 M.	3	2.00	19.91	C
15	12:00 M. – 3:00 P.M.	3	2.33	21.08	R
16	3:00 P.M.– 6:00 P.M.	3	2.00	21.50	R
17	6:00 P.M.– 9:00 P.M.	3	2.00	21.91	R
18	9:00 P.M.–12:00 N.	3	2.33	22.45	B
19	12:00 N. – 3:00 A.M.	3	2.00	23.83	A
20	3:00 A.M.– 6:00 A.M.	3	2.00	25.12	A
21	6:00 A.M.– 9:00 A.M.	3	2.33	25.52	B
22	9:00 A.M.–12:00 M.	3	2.00	26.77	C

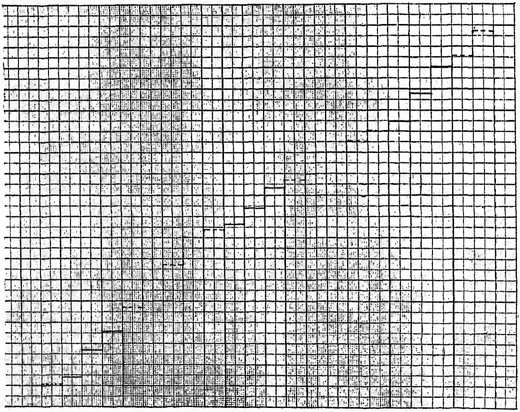

FIG. 9.

the periods between 9 P.M. and 6 A.M. It is impossible to derive an accurate $\frac{\text{day rate}}{\text{night rate}}$ ratio from so short an experiment. An arrangement for automatically taking out the used and putting

TABLE IX

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	8:00 P.M.–6:00 A.M.	10.00	4.00	3.36	A
2	6:00 A.M.–8:00 P.M.	14.00	3.70	3.59	A
3	8:00 P.M.–6:00 A.M.	10.00	5.10	3.59	A
4	9:15 A.M.–8:00 P.M.	10.75	2.23	4.18	A
5	8:00 P.M.–6:00 A.M.	10.00	3.30	4.18	A
6	6:00 A.M.–8:00 P.M.	14.00	2.85	4.47	B

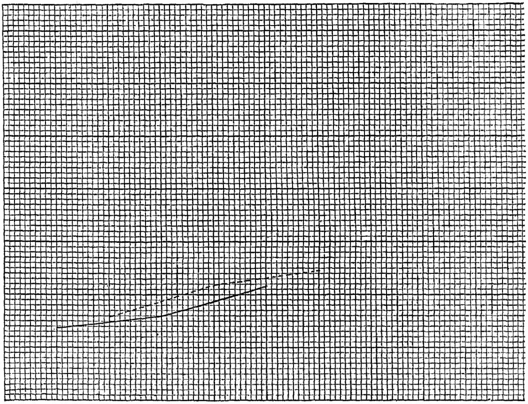

FIG. 10.

in the new Meyer's tubes will materially reduce the personal labor involved in the observations. The striking feature of the curve is the great irregularity of the single observations. Obviously it will be necessary to make measurements of the atmospheric conductivity simultaneously with those of carbon dioxide evolution in order to gain more light on these observations.

(8) Inserting the deionizing tube (p. 382), the other conditions were the same as in 7. The experiment ran from May 28 to 31. The results are given in table VIII and fig. 9. Here the rate shows a very much more regular increase.

(9) In order to determine whether the daily variation in carbon dioxide evolution was restricted to plants or whether it took place also in animals, an experiment (p. 383) was made with ten beetles,[11] placed in flask *R* on fine asbestos fiber soaked in water. Twice during the course of the experiment 2 cc. of water were run onto the asbestos. To all appearances, the beetles behaved perfectly normally during this time. The experiment ran from June 3 to 6. The results are given in table IX and fig. 10. $\frac{\text{Day rate}}{\text{Night rate}} = 1.099.$

Discussion

Although it is difficult to derive an exact $\frac{\text{day rate}}{\text{night rate}}$ ratio with material in which the respiratory activity is rapidly rising or falling, the following in a sense summarizes those of the foregoing experiments which ran for a longer time:

Material	$\frac{\text{Day rate}}{\text{Night rate}}$ normal air	$\frac{\text{Day rate}}{\text{Night rate}}$ deionized air
Onion	1.150	.....
Wheat	1.042	1.010
Wheat	1.091	1.014
Beetles	1.099	.....

There is at present no satisfactory explanation of these facts. The phenomenon of atmospheric ionization is undoubtedly exceedingly complicated, and perhaps at first glance far removed from our present conceptions of climatological factors of physiological importance. However, in view of the fact that ionization of the atmosphere is indicative of important chemical changes in the gases of the atmosphere, a physiological response to these changes is at least to be expected. These chemical changes, no doubt, are of such a nature as to affect the valency or activity of the atmospheric gases, in this case more especially the oxygen.

[11] The material (*Leptinotarsa 10-lineata*) was kindly loaned by Mr. John Sinclair, in charge at Tucson of the investigations of Professor W. L. Tower.

For example, the formation of ozone from molecular oxygen prerequires at least a partial dissociation of the oxygen molecule. It is interesting to note that in Vienna a relation of atmospheric ionization to the amount of ozone was established, the latter increasing with the former.[12] From the work of J. J. THOMSON[13] we know that the chemical effects produced by light are due to the emission of corpuscles from some of the atoms of the illuminated substance. Valency, under this conception, depends upon the relative ability of the atoms to eject or attract corpuscles. Now, it is of especial importance to note that BACH,[14] in elaborating the investigations and theories of MORITZ TRAUBE with especial reference to biological oxidative processes, comes to the conclusion that it is the partially dissociated oxygen (—O—O—) which combines with the oxidizable substance. Furthermore, C. ENGLER,[15] who with his co-workers has done a great deal to extend our knowledge of autoxidation, lays great stress upon the idea that for autoxidative processes the dissociation or liberation of free valencies in the oxygen molecule is necessary, and in this way he explains the accelerating influence of light and heat on oxidative processes. This very brief statement shows, I believe, that the air may possess higher "oxidative power" during the hours of illumination than during darkness.

In the foregoing pages the term respiratory activity has been used in a very general sense, and with special reference to aerobic respiration. There is, of course, little reason for supposing that the ionization of the atmosphere in any way affects the first stages in the katabolic processes, the breaking down or splitting of complex chemical substances. It is probably only in the oxidative processes that the action of the air plays a rôle. In what stages in the series of changes involved in this highly complicated process oxygen

[12] KAEHLER, KARL, Luftelektrizität. p. 56.

[13] THOMSON, J. J., The conduction of electricity through gases. Cambridge. 1913 (p. 290).

[14] BACH, M., Du rôle des peroxydes dans les phénomènes d'oxydation lente. Compt. Rend. Acad. Sci. 124:951–954. 1897.

[15] ENGLER, C., and WEISBERG, J., Kritische Studien über die Vorgänge der Autoxydation. Braunschweig. 1904.

KASTLE, J. H., The oxidases and other oxygen-catalysts concerned in biological oxidations. U.S. Hygienic Lab. Bull. **59**:9–30. 1910.

enters, is as yet not definitely established; nor can the formation of carbon dioxide be attributed entirely to the oxidative action of the oxygen. We know, however, that the total rate of respiration is greatly influenced by the accumulation or removal of the end products of the first stages in the series. Even assuming, then, that the only function of the oxygen is the removal of these end products, as is maintained by many physiologists, the more rapid oxidation of these substances would result in an increased total rate, and hence increased total amount of carbon dioxide evolved. This higher rate of oxidation, and consequent greater carbon dioxide evolution during the hours of sunlight, could be accounted for by the increased "oxidative power" of the air during this time. That no increased respiratory activity can be obtained with the use of artificial sources of light (except possibly the quartz mercury vapor lamps) is but natural, for light from these sources has no influence on the atmosphere.

The factors influencing atmospheric ionization should be briefly mentioned. Water vapor and high relative humidity have been found to decrease greatly the values; observations made at high altitudes usually show higher values than those made at about sea level. It is to be expected, therefore, that the respiratory activity in the arid and high regions, as, for instance, at Tucson, would be higher and show greater day and night variations than in a moist climate and at sea level.

Finally, it should be stated that the day and night variations as reported in this notice are not of great magnitude, and can be detected only by careful and rather prolonged experiments. The phenomenon is none the less important, however, when considered in its broader physiological bearing. The development of this hypothesis will naturally require many more experiments. Work is now in progress with still finer temperature regulations and on a larger variety of plants and animals. It is my intention to make simultaneous atmospheric conductivity measurements, and to study the effect of air artificially ionized by means of Roentgen rays or radium.

Desert Laboratory
Tucson, Ariz.

THE MEDULLARY RAYS OF CEDRUS

M. A. Chrysler

(with seven figures)

In those conifers the medullary rays of which are provided with marginal cells, it is commonly found that marginal tracheids in the xylem region of a ray are conterminous with the so-called "erect cells" in the phloem region of the ray. Such a condition was figured for *Pinus sylvestris* by Strasburger in his notable contributions to the anatomy of the conducting system (5), and the figure is copied in his textbook (6). It has in fact been claimed by more recent writers (7) that the marginal tracheids and the erect cells are corresponding structures, just as are ordinary tracheids and sieve tubes. It was accordingly with some surprise that the writer observed, in the course of a study of the origin of erect cells (1), that in the genus *Cedrus* erect cells were conterminous with parenchyma cells in the xylem region of a ray. An insufficient supply of material was at that time available, but recently this lack has been liberally supplied through the kindness of Miss R. Holden of Cambridge University, Mr. R. I. Lynch, Director of the Cambridge Botanical Garden, Professor E. C. Jeffrey of Harvard University, and Mr. H. N. Lee, formerly of the same institution. From these sources material of all three species of *Cedrus* and of the related monotypic genus *Pseudolarix* has been received, and it is now possible to give an interpretation of the anomalous features of *Cedrus* wood, and to offer evidence as to the relationships of the genus.

In a general way the wood of *Cedrus* bears much resemblance to that of *Abies*, but differs in having more numerous resin cells at the outer edge of annual rings, and in showing ray tracheids, which are, however, mixed with marginal parenchyma. Marginal cells are typically much less abundant than in *Pinus* and other Pineae; in *Cedrus* some rays lack marginal cells, while others are devoid of such cells for considerable distances. Strasburger (5) has called

attention to the fact that in this genus marginal tracheids occur with uncertainty, and concludes that they are generally more abundant in the mature trunk than in young branches, although they are sometimes scarce in the trunk.

A study of young stems and roots, and of older specimens where the phloem region is preserved, shows that when marginal cells are present in the xylem they are continuous with erect cells in the phloem, but that the latter are frequently present when the xylem

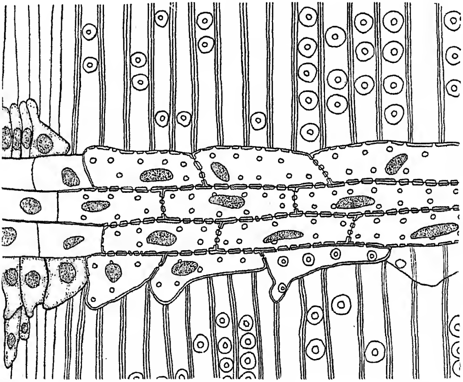

FIG. 1.—*Cedrus deodar*, stem; radial section through twelfth growth ring of xylem, with cambium and a small portion of phloem; in this and the succeeding figures the axis of the stem (or root) lies toward the right; ×290.

has no marginal cells. In such cases a triangular cambial cell occurs at the edge of the ray, as is shown in fig. 1, taken from a twelve-year old branch of *Cedrus deodar*. But in cases where such a medullary ray can be traced through several annual rings, it may generally be made out that scattered marginal cells occur, especially at the end of a year's growth, as may be seen in fig. 2, which represents a continuation of the section shown in fig. 1. Both margins of the ray in question show not only the scattered occur-

rence of the marginal cells, but the mixture of tracheids and parenchyma cells constituting the marginal row. The parenchyma of the marginal rows, like that of the central rows, is more or less completely filled with starch grains, which have been omitted from the figures for the sake of clearness. In some instances the marginal cells are practically restricted to the region of the end of an annual ring, and in such cases the marginal cells are entirely of a parenchymatous nature, as is shown in fig. 3, which is taken from

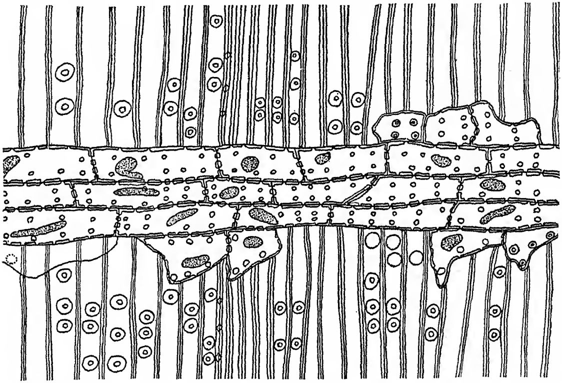

FIG. 2.—*C. deodar*, stem; a continuation of fig. 1, as may be seen from the presence of a shadowy cell on the lower margin of the ray in both figures; ×290.

near the cambial region of a root of *C. libani*. In this and other figures is to be seen the unusual shape of the marginal cells, which are triangular or pointed or tailed. It will be recalled that in most genera which possess marginal cells these are rectangular cells elongated in the radial direction, as are the central cells of a ray.

The lower margin of the ray shown in fig. 2 contains one of the shadowy cells or "ghosts" referred to by THOMPSON (7, 8) as characteristic of *Abies*, and considered by him to represent an

advanced stage of degeneration of marginal cells. These "ghosts" are of very frequent occurrence in the three species of *Cedrus*.

Another common feature of the marginal row is shown in fig. 4, from a root of *C. libani*, where a solitary cell containing rhombic crystals of calcium oxalate appears at the end of an annual ring, a situation which is quite the rule, though not invariable. Crystal-containing cells may also be found among the erect cells of the phloem region of a ray, as is shown in fig. 5. The occurrence of crystals in the phloem parenchyma is universal in *Cedrus*, as in

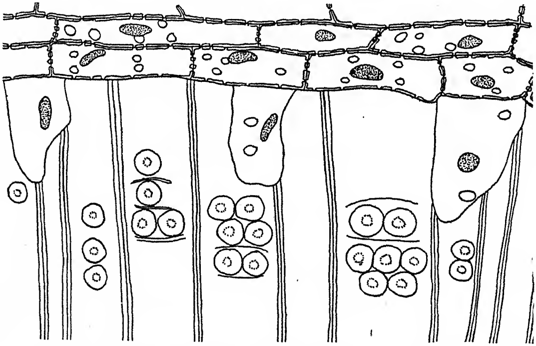

FIG. 3.—*C. libani*, stem; part of a ray traversing three growth rings, at the limit of each of which occurs a marginal parenchyma cell; ×290.

many other genera of conifers. Since calcium oxalate is an end product of katabolism, cells which are charged with it must be regarded as having passed their active or functional stage. This observation together with others here recorded indicate that the marginal cells are in process of degeneration and disappearance.

In order to determine if possible the order of appearance of the structures found at the margin of a ray, serial sections of young stems and roots have been studied. Material of seedlings has not been available, so that the study has been confined to roots and branches of an age of six to seven years. In fig. 6 is shown part of

the fifth and sixth annual rings of a branch of *C. libani*. It will be noticed that on the upper side of the ray the first marginal cell is a tracheid, followed at once by parenchyma, and on the lower side a tracheid is followed by a shadowy cell or "ghost," which still shows a faint bordered pit, and is in turn followed by a row of parenchyma cells. It must be admitted that in some cases the first cells to appear on the margin of a ray are parenchyma cells, but the figure shows the prevailing condition. As far as such observations afford evidence, they indicate that marginal tracheids antedate marginal parenchyma, and where only parenchyma is

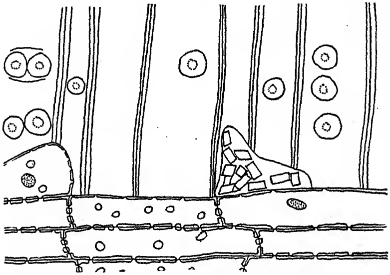

FIG. 4.—*C. libani*, root; a crystal-bearing cell takes the place of starch-bearing parenchyma at the limit of growth ring; ×290.

present it may be inferred that the earlier stages have been passed over.

In a number of cases it has been observed that marginal parenchyma cells are closely associated with resin cells. It will be recalled that in the genus *Cedrus* the resin cells occur on the outer face of the summer wood, and that it is at the limit of an annual ring that marginal parenchyma is most frequent. Fig. 7 shows the close relation of a row of resin cells to parenchyma cells of a ray in a branch of *C. atlantica*. It will be observed that the resin cell in view does not pass behind the ray, but that its end abuts against a marginal parenchyma cell which lies at the outer edge of a layer of summer wood, giving the appearance of a row of resin cells

forming a continuation or outgrowth of a marginal parenchyma cell. Such appearances are fairly common in all three species of *Cedrus*. These observations may throw some light on the origin of the marginal parenchyma. THOMPSON (8) in his account of the marginal parenchyma which occurs sparingly in *Abies* suggests that these cells have arisen in connection with the demand for food storage which occurs at the close of the growing season. The close association of marginal parenchyma with resin cells in *Cedrus* suggests, however, that the two structures may have arisen at the same

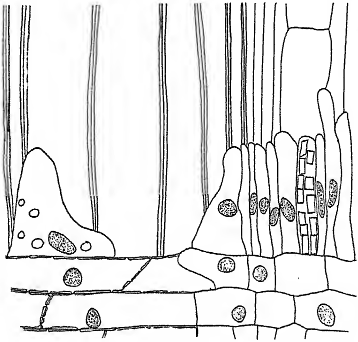

FIG. 5.—*C. libani*, root; one of the erect cells bears crystals; ×290.

time, or even that the vertically elongated marginal parenchyma cells gave rise to rows of resin cells. Of the different positions occupied by resin cells, the terminal position found in *Cedrus* appears to be the original one; at any rate, the plastic materials for supplying such cells are more abundant at the close of the growing season than at any other time. If we consider a marginal cell just cut off from the cambium, it is easy to see that if such cell is in contact with a resin cell which is being supplied with material from the medullary ray, the marginal cell will have a tendency to remain alive rather than thicken its wall and die, that is, develop into a marginal tracheid.

From a number of the observations here recorded the inference seems unavoidable that marginal cells in the genus *Cedrus* are in a vanishing condition. Such observations are: the scattered occurrence of marginal cells, varying from an almost continuous row to occasional cells; persistence of the erect cells of the phloem in line with a few scattering marginal cells on the xylem portion of a ray; the tapering or tailed shape of the cells; occurrence of

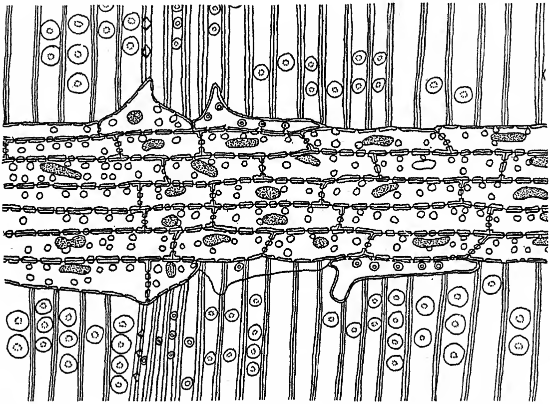

FIG. 6.—*C. libani*, stem; part of the fifth and sixth growth rings, showing earlier appearance of ray tracheids than parenchyma; ×290.

crystal-bearing cells in place of tracheids or starch-bearing parenchyma; occurrence of shadowy cells or "ghosts"; the capricious occurrence of marginal tracheids, as pointed out by STRASBURGER. If then the marginal cells are disappearing, *Cedrus* would appear to show reduction from some such genus as *Pinus* or *Picea*. The foliage of *Cedrus* suggests a relation to *Pinus*, while the cone bears more resemblance to that of *Abies*. The relation to *Pinus* is further indicated by the observation of JEFFREY (2) that when traumatic resin canals are induced in *Cedrus* they occur in both

the vertical and the horizontal plane, while in *Abies* they occur only in the vertical plane. Moreover, resin canals occasionally occur in the xylem of the cone in *Cedrus*, but not in *Abies*.

The foregoing facts go to show that *Cedrus* is in many respects intermediate between *Pinus* and *Abies*. That *Abies* stands farther

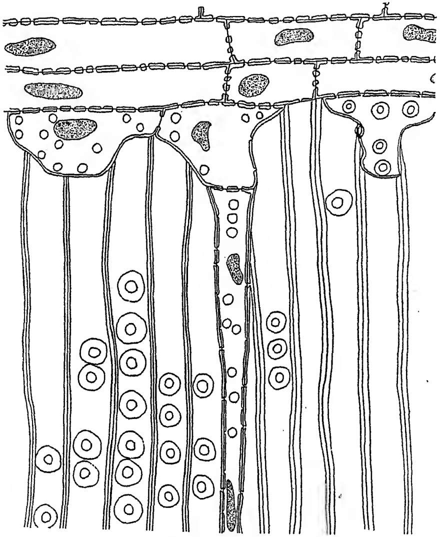

FIG. 7.—*C. atlantica*, stem; at the junction of two growth rings a row of resin cells forms a continuation of a marginal cell of the ray; ×425.

down than *Cedrus* in this reduction series is indicated by the fact that it is mostly in connection with wounds that marginal parenchyma occurs in *Abies*, while such cells occur normally in *Cedrus*. The following evolutionary stages in ray structure are indicated:

1. Medullary rays typically provided with marginal row of cells, which are tracheids: *Pinus*, *Picea*.

2. Marginal row consists of tracheids, but these are replaced by parenchyma at the limit of annual rings: *Cedrus*.

3. Many of the marginal tracheids have been replaced by parenchyma, or have degenerated into ghosts, or have disappeared: *Cedrus*.

4. Marginal cells typically absent, but occur sporadically, especially as a result of injury: *Abies*.

Cedrus shows itself to be a particularly plastic genus, not only in the lack of uniformity of its ray structure, but in its response to wounding, as shown by JEFFREY (2). It would be interesting to ascertain whether wounded material shows reversionary stages in the rays, but my material affords no evidence on this point. In marked contrast to *Cedrus* in this respect is the nearly related *Pseudolarix*. In no part of this plant which has come under my observation has any appearance of ray tracheids been observed, and JEFFREY (*loc. cit.*) remarks upon the absence of wound reactions.

The foregoing observations afford no support to the contention of PENHALLOW that marginal tracheids have been derived from parenchyma. Nor can we agree with his statement (4, p. 107) "the rare occurrence of tracheids in *Thuja*, etc., is to be interpreted as the first evidence of a tendency in development which is only fully realized at a later period." Since these words were written evidence has been accumulating which shows that the series must be read in the opposite direction, and that the sporadic occurrence of ray tracheids in the Cupressineae represents the last stage in disappearance of these cells. The chief evidence in this connection has been supplied by JEFFREY in his study of wound reactions, e.g., in *Cunninghamia* (3) and the observations on the rays of *Cedrus* point in the same direction. Further, there are physiological grounds for opposing the view quoted above, for it is easy to see how a complete row of marginal tracheids can function in carrying water radially, but a few scattered tracheids on the margin of a ray, e.g., of *Thuja*, must be entirely useless, and hence are better regarded as vestigial structures which point back to the time when the ancestors of *Thuja* had a functional row of marginal tracheids. Again, the writer has previously shown (1) that in *Juniperus*, a genus which like *Thuja* shows occasional marginal tracheids, the latter are conterminous with erect cells of the phloem, and erect cells sometimes occur where no marginal tracheids are

to be seen. This appearance is most readily interpreted as a persistence of marginal cells in the phloem after they have disappeared from the xylem.

Summary

1. The medullary rays of *Cedrus* are provided with a margin which varies greatly in composition, being made up of tracheids and parenchyma in varying proportion, or devoid of marginal cells for considerable stretches.

2. Marginal parenchyma when present occurs at the limit of annual rings, and may also extend beyond this point so as to be more plentiful than ray tracheids.

3. The constant occurrence of marginal parenchyma cells at the limit of annual rings, and their close connection with resin cells, indicates that parenchyma has replaced tracheids in connection with secretion of the so-called resin.

4. The marginal cells in *Cedrus* show distinct evidence of being in a degenerating condition.

5. The medullary ray structure confirms the view that *Cedrus* stands intermediate between *Pinus* and *Abies*.

University of Maine
Orono, Me.

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MICROTECHNICAL METHODS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 203

W. J. G. LAND

An improved method of replacing the paraffin solvent with paraffin

In paraffin imbedding the almost universal custom when replacing a paraffin solvent with paraffin is to add from time to time small pieces of paraffin to the solvent, until at room temperature no more paraffin is dissolved. Then the container is either placed in a low temperature oven or on an oven of higher temperature and paraffin added until the new saturation point is reached. The container is then placed in an oven having a temperature one or two degrees above the melting point of the paraffin and more paraffin is added. When the last paraffin has melted the mixture is poured off and replaced with pure melted paraffin. The object in repeatedly adding small quantities of paraffin is to prevent a too rapid increase in the density of the xylol-paraffin or whatever solvent is used for paraffin.

Objects to be imbedded, being heavier than xylol, sink to the bottom. Paraffin, which is heavier than xylol, also sinks, with the result that the objects are almost immediately surrounded by a dense layer of dissolved paraffin, thereby defeating the end sought by a gradual addition of paraffin. It has repeatedly come to the notice of the writer that much of the damage to delicate plant tissues takes place during the replacement of the solvent by paraffin. Many otherwise careful workers are particularly careless at this point.

In order to prevent the object from coming into immediate contact with the dense xylol-paraffin solution, a piece of wire gauze is bent in such a manner that it forms a support 2–3 cm. above the object, and xylol sufficient to rise 1–2 mm. above the support is added. The container (a shell or bottle) is then filled with blocks

of paraffin, corked, and set aside and not disturbed until the saturation point is reached. The container is next placed on the oven until the new saturation point is reached, next in the oven, and imbedding is proceeded with in the usual manner. Even here, when dealing with delicate plants, the xylol-paraffin is poured off only to the wire gauze and the container again filled with blocks of paraffin.

By this method paraffin is slowly dissolved, and as it descends is slowly and uniformly diffused through the xylol, thus preventing, in a large measure, damage to the object by rapid changes in density. It takes longer to reach the saturation point than when solid paraffin is permitted to fall to the bottom of the container, but little plasmolysis results.

Using delicate liverworts for test objects it was found that no deformation of tissue took place, except that which can be accounted for by the excessively large coefficient of expansion (0.00027854) of paraffin. The deformation caused by paraffin in an artificial cell was found to be exactly the same as is always present in plant cells when the paraffin is quickly cooled.

Many workers use a very close series of alcohols in dehydrating and a similarly close series in replacing alcohol with the paraffin solvent, and then undo all their careful work by indiscriminately adding paraffin to the solvent.

A method of fixing paraffin ribbons to the side with certainty

Albumen fixative, which is almost universally used to fasten paraffin ribbons to the slide, has many excellences and a few disadvantages. Among the latter is the property of coagulating when subjected to moderate heat, and in consequence losing its adhesive quality. Because of this it is sometimes impossible to use sufficient heat to straighten refractory or much wrinkled ribbons, especially if paraffin melting at 58–60° is used. Again, it is almost impossible to fix sections of certain refractory plants to the slide, even if the ribbons are first straightened by floating on warm water and then transferred to an albumen coated slide and allowed to dry without heating. This is particularly true of sections of antheridial and archegonial heads of some mosses and of the strobili of *Selaginella.*

In anatomical work with seedlings and sporelings it is necessary to have an absolutely unbroken series, extending sometimes over many slides, in which the loss of a single section would destroy the value of the entire series.

The well known principle that most colloidal substances, when treated with a solution of some salt of chromium, exposed to light and dried, become insoluble in water, was utilized with complete success. The modern photographic processes, such as printing in pigmented gums and gelatin, photogravure, etc., are based on this property of bichromated colloids.

In the Hull Botanical Laboratory the writer and his students first tried Le Page's liquid glue thinned to the consistency of albumen fixative and made slightly yellow by dissolving a small quantity of potassium bichromate in the thinned glue. The slide was smeared with a thin coating of the bichromated glue and dried in the light. Later a solution of gum arabic was tried with even better results.

The present practice is to spread a few drops of a 1 per cent solution of gum arabic on the slide, taking care to see that every portion of the surface is covered, and flood the slide with water made slightly yellow by dissolving in it a few crystals of potassium bichromate. The ribbons are then straightened out on the slide by means of heat, the excess solution drained off, and the preparation put aside in the light to dry. A very short exposure to light is sufficient to render the gum insoluble in water. After the slides are thoroughly dry they are treated in the usual manner.

In heating the slide to straighten out the ribbons no special precaution, such as necessary with albumen fixative, need be taken, since gum arabic does not lose its adhesive power at temperatures below the melting point of the hardest paraffin ever used in imbedding. The paraffin in the ribbon may even be melted without lessening in the slightest the adhesive property of gum arabic.

When a large number of slides are to be made it is very convenient to mix the gum arabic and the potassium bichromate solutions and flood the slide with the mixture. The solutions should be mixed immediately before using, since the mixture does not keep.

Albumen fixative is much improved if, instead of water, the bichromate solution is used.

In the foregoing process the strength of the bichromate solution seems to be immaterial. If it is stronger than 1 per cent, crystals will appear when the preparation dries. These crystals do no harm, since they never appear in the sections, but they leave unsightly spots on the glass. In practice a 0.2 per cent bichromate solution will be entirely satisfactory. The writer does not make a solution of definite proportions, but adds enough potassium bichromate crystals to make the water pale yellow. A very small quantity of a salt of chromium is sufficient, in the presence of light, to render gum and gelatin insoluble in water.

Imbedding in gelatin

In preparing hard woods for sectioning it is the custom to soften in hydrofluoric acid and imbed in celloidin. Since this process involves dehydration, some refractory woods become unmanageable when sectioning is attempted.

In connection with the work of M. A. Brannon on the extremely hard stems of plants which had been submerged by the rising waters of the Salton Sea and then exposed when the water receded, a method of imbedding in gelatin was devised by the writer and successfully used. Many of these stems were decorticated, some partially macerated, and all were excessively hard.

Gelatin is soaked in water until no more is taken up, the excess water drained off, and the gelatin liquefied by heat. Pieces of wood previously softened in water, or if necessary in hydrofluoric acid, are placed in the melted gelatin for some hours. Small blocks of hard wood to serve as supports in the microtome are also placed in the melted gelatin. The blocks to be sectioned are properly oriented in a gelatin matrix on the supporting blocks, cooled to set the gelatin, and plunged into strong formalin to harden the gelatin. In cutting the knife is flooded with water.

The advantages of this method are that no dehydration is necessary; that the process is very rapid; and that partly disintegrated tissues are held in place. In careful hands sections of hard woods can be cut as thin as is possible by the celloidin process.

Softening refractory material imbedded in paraffin

Plant material, especially if much starch is present, will not cut readily in paraffin. For complete infiltration with paraffin dehydration must be thorough, and a corresponding hardening of tissues results.

As is well known, paraffin is pervious to water. If imbedded material impossible to cut without fragmentation or tearing of the sections be stored in water for some weeks or months it will in most instances section readily. The effect of water on imbedded material will be most strikingly shown if an attempt is made to cut the gametophyte of some of the cycads at or just after fertilization of the egg immediately after imbedding, and again after the paraffin cakes have lain for some months in water. Dormant embryos of *Helianthus* which will not ribbon immediately after imbedding give unbroken ribbons after the paraffin block has been soaked for some weeks in water. The writer stores in water all paraffin containing hard material.

A method of cleaning cover glass

In attempting to clean cover glasses 50–60 mm. long by wiping with a cloth after they have been freed from the cleaning fluid, many are broken even with the most skilful and practiced handling. Also it is almost impossible to have them free from lint.

In the writer's practice cover glasses are placed in the usual cleaning fluid used for laboratory glassware, a mixture of sulphuric acid and potassium bichromate, rinsed under a tap to completely remove the acid, placed while wet in alcohol, and finally completely submerged in 95 per cent alcohol until wanted. To use, the cover glass is slowly withdrawn from the alcohol so that a minimum film of alcohol will remain on the glass, one end touched to a piece of absorbent paper free from dust to remove the drop of alcohol, touched to a flame, and when the alcohol has completely burned off placed while warm on the slide.

This method is very rapid and gives beautifully clean *cover glasses* with practically no breakage. If the cover glass is drawn from the alcohol so slowly that a very thin film remains, a small crack in the cover glass will not spread.

University of Chicago

EFFECT OF MOISTURE CONTENT OF A SANDY SOIL ON ITS NITROGEN FIXING POWER

C. B. Lipman and L. T. Sharp

Since attention to the well being of the beneficial soil bacteria is admittedly a vital factor in the maintenance of soil fertility, it becomes as important a question to determine the relation of a soil's moisture content to its bacterial activity as to ascertain the relation thereof to the growth of the plant itself. Relatively little work has thus far been carried out along such lines, and such data as we have bear, so far as we are aware, almost wholly on the relation of the soil's water content to ammonification, nitrification, and dentrification. Nothing but the one investigation below named, which is possessed of any cogency, has thus far come to our notice which deals with the aspects of the same question with regard to nitrogen fixation. The writers therefore present in this paper a series of interesting results bearing on the subject in question which were obtained in the course of some of their studies on the natural nitrogen fixing flora of soils.

The history of the general subject of the relation of soil moisture content to bacterial activity, as above intimated, records but few investigations. Those possessed of any cogency here are those of Engberding,[1] Lipman and Brown,[2] Coleman,[3] Deherain,[4] and Krainsky.[5] The first named investigator found that the number of bacteria increased with the water content of the soil until the latter reached 80 per cent of saturation, and that it decreased when moisture was supplied in greater quantities. Lipman and Brown found in a neglected clay loam soil that ammonification increased with the increase in water content even up to

[1] Cited from Exp. Sta. Record 21:1909, p. 620.

[2] N.J. Exp. Sta. Rpt. 1908, p. 105.

[3] Cent. Bakt. 20^2:1907–1908, pp. 401 and 484.

[4] Compt. Rend. 125:1897, p. 282.

[5] Cent. Bakt. 20^2:1907–1908, p. 732.

35 per cent of the weight of the soil. They found, however, that nitrification was most active in the same soil with a moisture content of 15 per cent, was only slightly less active with 10 per cent of moisture, and even quite appreciable with 5 per cent of moisture. COLEMAN in working with a loam soil found nitrification in it most active with a moisture content of 16 per cent, thus agreeing with the results of LIPMAN and BROWN. Contrary to the results of the latter, however, COLEMAN obtained marked reduction in nitrification when the moisture content of the soil was reduced to 10 per cent, but again obtained similar results to those of the other investigators when the moisture content of the soil was increased to 26 per cent. DEHERAIN'S findings, in work with the nitrifying flora, were in harmony with those of the foregoing investigations on nitrification. KRAINSKY in working with the nitrogen fixing flora found nitrogen fixation considerable even in soil with less than one-fourth of the optimum moisture content.

In our work on nitrogen fixation a light sandy soil from a walnut grove in Anaheim, California, was employed, and the natural nitrogen fixing flora thereof studied in its relations to moisture. The soil culture method was used in which 50 gram portions of soil were mixed in tumblers with 1 gram of mannite and water added in varying quantities as indicated in the table below. The mixture was stirred with a sterile spatula, the tumblers covered with Petri dish covers and incubated at 28°–30° C. for 21 days. Other explanatory data along with the amount of nitrogen fixed in the cultures with varying quantities of moisture are given below in table I.

It is evident from the figures in the foregoing table that nitrogen fixation in a sandy to sandy loam soil by means of its natural flora and under optimum temperature conditions takes place most actively with a water content varying from 20 to 24 per cent based on the air dry weight of soil, or 22.5 to 26.5 per cent based on the water free soil. Even with 28 per cent of moisture (air dry basis), nitrogen fixation manifests an activity but little less potent than that just mentioned. With a moisture content of 32 per cent a marked decrease in nitrogen fixing power of the soil is evident, and a still greater decrease is noted with the largest water content employed, namely 36 per cent. None the less, it should be noted

that even at the latter moisture content notable nitrogen fixation occurs in what is virtually a saturated soil.

TABLE I

INFLUENCE OF MOISTURE CONTENT ON NITROGEN FIXING POWER OF SANDY SOIL

Culture no.	Per cent moisture air dry basis (hyg. moisture 2.5 per cent)	N found mgs.	N fixed per gram of mannite mgs.	Av. N fixed per gram of mannite mgs.
1	0	31.15	0	0
2	0	31.15	0	0
3	4	31.85	.70	.88
4	4	32.20	1.05	
5	8	34.65	3.50	3.68
6	8	35.00	3.85	
7	12	37.10	5.95	5.95
8	12	37.10	5.95	
9	16	36.75	5.60	5.95
10	16	37.45	6.30	
11	20	39.20	8.05	8.05
12	20	39.20	8.05	
13	24	39.55	8.40	8.05
14	24	38.85	7.70	
15	28	38.15	7.00	7.18
16	28	38.50	7.35	
17	32	36.40	5.25	4.55
18	32	35.00	3.85	
19	36	33.95	2.80	2.98
20	36	34.30	3.15	

With small amounts of moisture in the soil some interesting results are obtained, also, as indicated in the table. Virtually no nitrogen fixation or very little takes place with a moisture content of 4 per cent (air dry basis), but a very marked increase occurs when

8 per cent is present, and amounts of moisture equivalent to 12 per cent (air dry basis) give about the same nitrogen fixation as 32 per cent moisture.

To comment on and summarize the interesting results above given we may make the following statements.

1. Nitrogen fixation by a soil's natural flora being the algebraic sum of the activities of several classes of bacteria both aerobic and anaerobic, it must follow that the greatest fixation of nitrogen will occur at a moisture content very favorable for the most active forms of nitrogen fixing bacteria, and yet not entirely unfavorable for the less potent forms, or vice versa. In the soil in question that point seems to lie between the limits of 20 per cent and 24 per cent of moisture (air dry basis). It would appear just to conclude from these data that the aerobic forms of nitrogen fixing bacteria do best with a 20 per cent moisture content (the optimum for that soil on a physical basis). At higher percentages of moisture up to 24 per cent the anaerobic forms become much more active, while the aerobic forms are depressed in their nitrogen fixing powers. This gives us two maxima of nitrogen fixation in that soil based on the moisture content or what appears from the table to be a curve which runs along at the same plane between rather wide limits.

2. The case of nitrogen fixation with respect to soil moisture content, therefore, would seem to be analogous to that of ammonification as studied by LIPMAN and BROWN as referred to above. In both cases the end products which are measured represent the algebraic sum of the activities of both aerobic and anaerobic organisms.

3. We find in confirmation of the findings of KRAINSKY, above mentioned, that nitrogen fixation is very active even with low moisture content of the soil. Thus, with a moisture content of only 8 per cent very considerable quantities of nitrogen are fixed, and, to judge from the excellent agreement between duplicate nitrogen determinations in our work, appreciable quantities of nitrogen are fixed with a moisture content of only 4 per cent.

4. Taken as a whole, the nitrogen fixing flora of the soil with which we worked, and which may be taken as a criterion for a large variety of sands and sandy loams, behave much more like the

ammonifying flora than like the nitrifying flora with respect to moisture. This is also in harmony with other results from the point of view of factors other than the soil moisture content, which one of us has obtained with respect to the behavior of these three groups of organisms.

5. We feel, as did DEHERAIN in his work cited above, that changes in the physical constitution of a soil will seriously modify the points of maximum and minimum bacterial activity with a given moisture content. But the more exact determinations of available moisture in all soils as advocated by BRIGGS will probably indicate but slight variations from the optimum and minimum moisture contents necessary for the activity of soil organisms as determined for the ammonifying and nitrifying flora by the investigators above named and for the nitrogen fixing flora by us.

LABORATORY OF SOIL CHEMISTRY AND BACTERIOLOGY
UNIVERSITY OF CALIFORNIA

BRIEFER ARTICLES

EVIDENCE FOR THE GENERAL DISTRIBUTION OF OXIDASES IN PLANTS

If the oxidases play the essential rôle in respiration which recent theories have attributed to them, they must be present in all living cells. Two types of tissues, however, have been reported to be without oxidases, namely, tissues markedly acid in reaction and some tissues said to contain large amounts of reducing substances.

Recently it has been pointed out by the writer[1] that in some of the most acid plant tissues, the citrus fruits, the reported absence of oxidases was due to the methods of investigation. By separating out the carpel sacs in such a way that the acid juice and the ferment were kept apart, as is normally the case in the living tissue, good oxidase reactions were obtained; whereas in former methods the tissue was ground in an attempt to extract the ferment, and the latter was then inhibited by the action of the acid.

Tissues reported to be free of oxidases on account of the presence of reducing substances include a few organs of some higher plants and the algae as a group (with but two definite exceptions), so far as they have been investigated.[2]

Examining a few representative forms from the algae with a number of reagents, results as indicated in the accompanying table were obtained. The material was freed from foreign matter as far as possible, ground, or in the case of the large brown forms simply torn up and added to the solutions of the reagent. The formation of oxidation products was then followed by color changes as compared with checks containing boiled material.

The results indicated in the table were further confirmed in the case of the filamentous forms by following the reactions in individual cells. The material was placed in a one-half per cent watery solution of paraphenylenediamine, or equal parts of one-half per cent solution of paraphenylenediamine and alpha napthol (Spitzer's reagent); and in each

[1] Bot. Gaz. 57:528. 1914.

[2] Atkins, Sci. Proc. Roy. Dublin Soc. 14:11; Duggar and Davis, Science N.S. 39:260. 1914.

case sufficient hydrogen peroxide to make the concentration 0.1 per cent. Results were similar in the two cases. In from one-half to ten minutes the oxidation products appeared in the form of minute dark granules exhibiting slow Brownian motion and distributed throughout

TABLE I

THE OXIDATION OF VARIOUS COMPOUNDS BY HYDROGEN PEROXIDE WHEN ACTIVATED BY JUICE EXPRESSED FROM LIVING ALGAE: (+) INDICATES A POSITIVE REACTION IN TEN MINUTES OR LESS; (−) INDICATES NO REACTION EVEN AFTER SOME HOURS OR ONE TOO FAINT TO BE CONSIDERED TRUSTWORTHY.

	Gum guaiac	Pyrogallol	Hydrochinone	Alpha napthol	Alpha napthol and Para-phenylenediamine	Para-phenylenediamine	Alpha napthol and Para-phenylenediamine hydrochloride	Para-phenylenediamine hydrochloride	Benzidine	Aloin	Phenol	Para-cresol	Sodium selenite
Blue-green													
Oscillaria sp...	−	−	−	+	+	+	+	+	−	−	−	−	−
Green													
Conferva sp....	−	−	−	−	+	+	−	−	−	−	−	−	−
Spirogyra sp...	−	faint	faint	−	+	+	+	+	+	−	−	−	−
Ulva sp.......	+*	+*	+*	−	+*	+	+	+	+	faint	−	−	−
Enteromorpha sp...........	−	−	−	−	+	+	−	−	+	−	−	−	−
Chaetophora sp.	−	−	−	−	+	+	+	+	+	−	−	−	−
Vaucheria sp...	−	−	−	−	+	+	+	+	+	−	−	−	−
Chara sp......	−	−	−	−	+	+	−	−	−	−	−	−	−
Brown													
Laminaria saccharina.......	−	−	−	−	+	+	+	+	+	−	−	−	−
Fucus sp......	−	−	−	−	+	+	+	+	+	−	−	−	−
Ascophyllum nodosum......	−	−	−	−	+	+	+	+	+	−	−	−	−
Red													
Chondrus crispus..........	−	−	−	−	+	+	−	−	+	−	−	−	−
Polysiphonia sp.	−	−	−	−	+	+	−	−	−	−	−	−	−

* Reaction with and without hydrogen peroxide.

the protoplasm. The size of the granules varied slightly in different species, but in all cases granules were found only in the protoplasm, never in the vacuole. In *Vaucheria* (and occasionally in *Spirogyra*) they showed a tendency to aggregate about the nuclei, though never about the chromatophores. In cells which had been boiled, however,

though the structure of the cell had not been seriously affected, no granules were formed, since the boiling had destroyed the ferment.[3]

An examination of the results summarized in the table shows that, so far as one is able to judge by color changes, the algae as a class possess a ferment capable of activating the oxidation of a limited number of compounds. In other words, it appears that this ferment is specific in its action. The condition is not surprising, inasmuch as it is characteristic, to a greater or less extent, of all ferments and has many parallels among the oxidases themselves.

From the uniformity of results with the forms examined, it is apparent that the oxidases are of general occurence among the algae. These, and the writer's observations on acid tissues, indicate that the oxidases are universally distributed in living plants, and that other cases of their apparent absence may be explained in ways similar to those herein discussed.—G. B. REED, *Laboratory of Plant Physiology, Harvard University.*

[3] A more detailed account of the formation of these granules in plant cells is soon to be published.

CURRENT LITERATURE

BOOK REVIEWS

The Salton Sea

The remarkable overflow of the Colorado River in 1905–1907, causing the submergence of a portion of the Cahuilla Basin, resulted in an expanse of waters known as the Salton Sea. At its maximum, this sea had a depth of 84 feet and an area of 410 square miles, but since the checking of the influx from the Colorado River in 1907, it has suffered an annual subsidence, due to the excess of evaporation over precipitation, averaging about 4.5 feet. These phenomena afforded excellent opportunities for ecological investigations of a unique character, which have been conducted by the Department of Botanical Research of the Carnegie Institution.[1]

The report begins with an account of the discovery, exploration, and geologic history of the Cahuilla Basin, prepared by the late WILLIAM P. BLAKE two years previous to his death in 1910. His connection with the exploration of the area extends from his discovery of the basin in 1853, when he was a member of the Williamson Expedition, to this last visit in 1906. Further details regarding the geographical features of the region are contributed by GODFREY SYKES, who includes reproductions of some of the earliest maps, beginning with one by CASTILLO made in 1541 and first published in 1770. E. E. FREE gives a sketch of the geology and a discussion of the two types of soil, the coarser composed of gravel and sand resulting from the decomposition of the granitic rocks *in situ;* the other, an alluvium of fine texture. Both are decidedly fertile, except for the local development of alkaline conditions. ROSS and VINSON provide a comparison of the chemical composition of the water at various intervals from 1906 to 1913, showing a close resemblance to that of ordinary sea water and an increasing concentration of salts with the present continuous recession. The behavior of micro-organisms in the brine is reported by G. J. PEIRCE, a small red chromogenic bacillus receiving particular attention. M. A. BRANNON, working at the Botanical Laboratory of the University of Chicago upon the action of the Salton Sea water on vegetative tissues, reports no evidence of petrifaction of woody tissues, but a decortication of woody plants submerged for a year or more, due to the enzymic action of bacterial organisms upon the tissues of the cambium region.

[1] MACDOUGAL, D. T., and COLLABORATORS, The Salton Sea. A study of the geography, the geology, the floristics, and the ecology of a desert basin. Carnegie Inst. Pub. 193. pp. 182. *figs. 4. pls. 32.* 1914.

Naturally, the floristics and plant ecology of the area receive the major portion of attention. S. B. PARISH sketches the history of its botanical exploration, from a botanical paper by Dr. C. C. PARRY in Emory's Report of the Survey of the boundary between the United States and Mexico, made in 1856. He presents an annotated list of indigenous and introduced species, the former including 8 trees, 23 shrubs, 10 suffrutescent plants, 30 perennial herbs, and 51 annuals. Only 7 species are endemic. A grouping is made into formations and associations, the halophytic and xerophytic naturally being the most prominent. Detailed sketches of some of the outskirts of the area have already appeared.[2] This analysis of the composition of the vegetation is continued in MACDOUGAL'S inquiry into its genesis, as shown by its re-establishment upon areas sterilized by submergence. He considers both the re-occupation of the strand left bare by the receding lake and that of sterilized islands emerging from the lowering waters. The changes as the aridity of the strand increases, the agencies effective in carrying seeds, and the invasion of new species are among the topics receiving attention, while a detailed history is given of various portions of the strands emerging from the waters. Among the pioneer forms, species of *Atriplex*, *Heliotropium*, *Sesuvium*, *Pluchea*, *Distichlis*, and *Suaeda*, together with *Prosopis pubescens*, *P. glaudulosa*, and *Salix nigra*, are found, but their abundance and survival differ at different points along the shore, and this could be to some extent related to the slope and character of the soil. The fact that 4 out of 60 species found upon the strand showed modifications of structure not observed elsewhere suggests the possibility that the changing conditions are resulting in the production of new species, and that similar series of changes in the past have been similarly productive.

The exactness of the present report and the abundance of its data also combine to make it a most valuable record for the future study of these as well as of other problems which may arise with the continual subsidence of the sea and the further development of the surrounding vegetation.—GEO. D. FULLER.

MINOR NOTICES

Flora of the Dutch West Indian Islands.—BOLDINGH[3] has published a second volume under the foregoing title, which deals with the islands of Curaçao, Aruba, and Bonaire. The present volume is divided into three parts, first Systematical, second Historical, and third Phytogeographical. The last part is subdivided into the following sections: (A) Orological, Geological, and Meteorological, (B) Distribution of the wild plants enumerated in the first part, (C) The vegetation of Curaçao, Aruba, and Bonaire. To this is added an

[2] PARISH, S. B., Sketches of the Colorado Desert. Plant World 17:122–130. 1914.

[3] BOLDINGH, I., The flora of the Dutch West Indian Islands. Second Volume. Curaçao, Aruba, and Bonaire. 8vo. xiv+197. *pls. 9. map 1.* Leyden: E. J. Brill. 1914.

alphabetical index to vernacular and their corresponding scientific names. The three islands represent an area of 860 square kilometers, or about 332 square miles, and possess a flora of 394 species distributed among 80 families from Polypodiaceae to Compositae. Those families predominating, as shown by the number of species recorded, are Leguminosae (41), Gramineae (35), Convovulaceae (25), Euphorbiaceae (24), Cyperaceae (23), and Compositae (20). One new species is described in each of the following genera: *Schizachyrium*, *Ficus*, *Pisonia*, *Kallstroemia*, *Bursera*, *Phyllanthus*, *Croton*, *Maytenus*, *Condalea*, *Casearia*, and *Melampodium*.—J. M. GREENMAN.

Applied botany.—KRAEMER[4] has accomplished a very laborious task for the benefit of students in technical schools, pharmaceutical and medical colleges, food analysists, etc. Although emphasizing the technical side of plants, he has included a basis of morphology and physiology, which should put the student, interested chiefly in the commercial aspect of plants, in touch with the scientific aspect. The seven chapters include the following subjects: Principal groups of plants, under which is given an outline of the plant kingdom; Cell contents and forms of cells; Outer and inner morphology of the higher plants; Botanical nomenclature, which is also a glossary of technical terms; Classification of angiosperms yielding economic products; Classification of medicinal plants; and Microscopic technique of reagents.

The book is a thesaurus of information, and as a book of reference should be of great service to botanists in general.—J.M.C.

North American flora.—The first part of volume 34 presents 50 genera of the Helenieae, all but 2 of them by RYDBERG.[5] The new genera proposed are *Nesothamnus* (type species, *Perityle incana*), *Leptopharynx* (type species, *Perityle Parryi*), *Pappothrix* (type species, *Laphamia rupestris*), *Amauriopsis* (type species, *Amauria dissecta*), *Cephalobembix* (type species, *Schkuhria neomexicana*), *Trichymenia* (type species, *Hymenothrix Wrightii*). New species are described also in *Venegazia*, *Psilostrophe* (3), *Baileya* (3), *Perityle* (5), *Laphamia* (2), *Loxothysanus*, *Bahia* (2), *Hulsea* (3), *Tetracarpum* (2), *Hymenopappus* (5), *Othake* (2), *Rigiopappus*, and *Chaenactis* (3).—J. M. C.

NOTES FOR STUDENTS

Toxic effects.—The observation that small traces of salicylic acid (o-oxybenzoic acid) in the presence of comparatively large quantitites of p-oxybenzoic acid have a deleterious effect on the growth of *Penicillium*, while both p-oxybenzoic and m-oxybenzoic acids serve as food, has led BOESEKEN and

[4] KRAEMER, HENRY, Applied and economic botany. 8vo. vi+806. *figs. 424*. Philadelphia (145 N. 10th St.): Published by the author. 1914. $5.00.

[5] North American Flora **34**: part 1. pp. 80. Carduaceae (Helenieae), by P. A. RYDBERG; *Baeria* and *Lasthenia*, by H. M. HALL. New York Botanical Garden, 1914.

WATERMAN[6] to investigate the behavior of these acids and numerous other compounds with reference to their toxic action and to their nutritive value. The substances investigated cover a wide range, including acids, alcohols, and chlorine and bromine derivatives of the hydrocarbons of the aliphatic series, and acids and their oxygen derivatives, phenols, and hydrocarbons of the carbocylic series. The work is not well organized, and the results lack quantitative value. The extent of growth of the fungus in the different solutions is indicated by + and −. The ranges of concentrations of the substances used are not sufficiently wide to permit the determination of inhibiting concentrations with any degree of exactness. Moreover, the concentrations used are entirely arbitrary and without reference to chemical properties of the substances. In some cases of slightly soluble substances (the higher fatty acids) the concentrations were not known. Some of the results appear unusual. It is known that the various species of blue molds are not particularly selective as to their food. It is surprising, nevertheless, to note that such substances as chloroform, carbon-tetrachloride, dichlor-brom-ethylene, and benzene are said to serve as nutrients for these molds. The question occurs whether the belated growth in flasks containing these somewhat volatile substances was anything more than the film of growth formed by *Penicillium* even on the surface of inert solutions to which no organic nutrients have been intentionally added. The authors believe that a general parallelism exists between the relative toxicity of the various substances and their partition coefficient in oil and water. They find, therefore, a satisfactory explanation of the different behavior of the substances in the theories of MEYER and OVERTON, both of which, it should be stated, however, were formulated with reference to chemically inert substances or, at most, very weakly basic organic substances (OVERTON).

The conclusions reached by the authors may be summed up as follows. In general, substances act as food or poison according to the magnitude of their partition coefficients in oil and water, those relatively very soluble in oil being highly toxic, and those with a low partition coefficient serving as food. Some substances (cetyl alcohol, higher fatty acids, napthalene) very soluble in oil but only slightly soluble in water serve as food, and by reason of their slight

[6] BOESEKEN, J., and WATERMAN, H., Over een biochemische methode ter bepaling van kleine haeveelheden salicylzuur naast een overmaot p-oxy benzoëzuur. Konink. Akad. Wetensch. Amst. 20^1:548–552. 1911.

———, Over de werking van eenige benzolderivaten op de ontwikkeling van *Penicillium glaucum*. *Ibid.* 552–567. 1911.

———, Over de werking van eenige koolstofderivaten op de ontwikkeling van *Penicillium glaucum* en hunne remmende werking in verband met oplosbaarheid in water en in olie. *Ibid.* 20^2:965–973. 1912.

———, Werking van in water gemakkelijk, in olie niet oplosbare stoffen op den groei van den *Penicillium glaucum*. *Ibid.* 1246–1251. 1912.

solubility in water are not toxic. Substances easily soluble in water act either as poisons or as nutrients according to their relatively higher or lower solubility in oil. Substances easily soluble in water and slightly soluble in oil act as nutrients, but not as poisons. In other cases, where this theory fails to explain the toxicity of substances, as with some dibasic acids easily soluble in water and scarcely soluble in oil, which nevertheless are toxic, the toxicity is attributed to the hydrogen ion. These exceptions, as well as a number of others (formic acid, etc.), go to show that the action of substances on organisms cannot be explained on the basis of any one characteristic of the substances. The hydrogen ion is toxic, but acetic acid possesses a toxicity far in excess of that attributable to its hydrogen ion. Cane sugar, regardless of its solubility in oil or in water, can be utilized only by those organisms which contain invertase.

The inhibiting and sometimes fatal effects which the accumulated products of metabolism exert on organisms producing them are matters of general observation. Different organisms vary much, however, in their behavior toward their own products. An interesting illustration of this difference of behavior is brought out by WEHMER[7] in his studies of the effect on *Penicillium variabile* and *Aspergillus niger* of acids accumulating in culture solutions upon which these fungi are growing. The behavior of these two organisms differs widely. In cultures of *Penicillium* on solutions containing ammonium sulphate, WEHMER observed inhibition of growth and ultimately death of the fungus as a result of the accumulation of free acid in the solution. This result does not come about in cultures in which potassium nitrate, ammonium nitrate, ammonium chloride, or ammonium salts of organic acids are the source of nitrogen. In the case of nitrates, both ions are consumed, although here also nitric acid accumulates in the cultures at first. Hydrochloric acid seems to be comparatively harmless to this fungus. In cultures of *Aspergillus niger*, also, acid accumulates in the solution when ammonium salts of inorganic acids are offered as sources of nitrogen, but in the course of a few weeks there is in all cases a diminution of the acidity of the solution. The diminution is most marked with sulphuric acid and least with hydrochloric acid. Growth is not injured by the temporary accumulation of acid, but spore production is inhibited. The acidity is due to the accumulation of inorganic acid and not to the production of organic acids. The author attributes the lowering of the acidity of the cultures to a neutralization of the acid by the products of the protein decomposition in the older parts of the mycelium. Thus the ammonia consumed during the early growth of the culture is finally

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[7] WEHMER, C., Selbstvergiftung in *Penicillium*-Kulturen als Folge der Stickstoff. Ernährung. Ber. Deutsch. Bot. Gesells. **31**:210–225. 1913.

———, Der Gang der Acidität in Kulturen von *Aspergillus niger* bei wechselnder Stickstoffquelle. Biochem. Zeitschr. **59**:63–76. 1914.

liberated and becomes available for the neutralization of the accumulated acid when through want of a carbon nutrient rapid growth is no longer possible.

KIESEL[8] has examined a large number of acids, mostly organic, and salts of some inorganic acids with reference to their toxicity to *Aspergillus niger*. The numerous data which do not permit of any brief generalizations are, like other data of this kind, of interest more from a toxicological than from a physiological standpoint. The author's conclusion that the toxicity of organic acids cannot be entirely ascribed to the dissociated part of the molecule is in accordance with the findings of other investigators. The mode of action of the dissociated part of the molecule has not thus far been explained. The author's hypothesis, that toxic action which cannot be correlated with chemical properties is associated with the degree of permeability of the protoplasm to the substances exerting such action, is interesting in the light of the researches of MYER and of OVERTON, but it lacks experimental evidence, for it has not been shown on the one hand that toxicity and power to penetrate the protoplast go hand in hand in such cases, and on the other hand it is self-evident that substances which do penetrate the protoplast cannot act as poisons. The author fails to find a regulatory depression of acidity of the medium, as has been reported by some investigators. With regard to the relative toxicity of the chlor-acetic acids, KIESEL's results harmonize with those of CLARK, who found that the introduction of one or two chlorine atoms into acetic acid increased its toxicity, but the introduction of a third chlorine atom lowered the toxicity.

The problem of antidotal action, or antagonism of one substance toward another, assumes special significance in the study of the toxicity of various substances to fungi, since the toxic substances whose effects are to be studied can rarely be used alone, but must usually be combined with other electrolytes or non-electrolytes requisite for the growth of mycelium and in most cases even for the germination of spores. Several papers dealing with this problem have appeared recently.

From this point of view BOESEKEN and WATERMAN[9] have studied the influence of a series of substances on the toxicity of boracic acid toward *Penicillium glaucum* and *Aspergillus niger*. The substances studied were glycerine, sorbite, dulcite, mannite, arabinose, xylose, glucose, levulose, mannose, rhamnose, galactose, maltose, lactose, raffinose, sucrose, p-oxybenzoic acid, protocatechuic acid, and gallic acid. Definite concentrations (usually 2 per cent) of these substances were used in connection with varying concentrations of the acid. The culture solutions were made with tap water, to which

[8] KIESEL, A., Recherches sur l'action de divers acides et sels acides sur le développement de l'*Aspergillus niger*. Ann. Inst. Pasteur **27**:391–420. 1913.

[9] BOESEKEN, J., and WATERMAN, H. J., Über die wirkung der Borsäure und einiger anderen Verbindungen auf die Entwicklung von *Penicillium glaucum* und *Aspergillus niger*. Fl. Microbiologica **1**:342–358. 1912.

ammonium chloride, potassium di-hydrogen phosphate, and magnesium sulphate had been added. The effect of the acid in combination with the different substances was estimated by the relative development of the cultures. It is noted that the antitoxic action of the substances examined is correlated with the ease with which they combine with the acid, as shown by conductivity changes of the mixtures. Those which combine most readily with boracic acid are also most effective as antidotes to the toxic action of the acid. The antagonistic action of these substances, therefore, is attributed to their power of holding the acid in combination.

Studies of a similar nature have been made by KUNKEL,[10] who investigated the antagonistic action with reference to *Monilia sitophila* between inorganic salts and such substances as peptone, starch, glucose, fructose, and galactose, which are frequent constituents of culture media. These substances all modify the toxicity of salts, but not to the same extent nor in the same order. The author believes that this modification of toxicity may in part be the result of reactions between the salts and the organic substances of the medium,[11] but there appears to be no constant correlation between reduction in the concentration of ions caused by the addition of nutritive substances and the toxicity of the medium. On this account the author believes that the food substances themselves have an influence on the organism by which it is enabled to endure higher concentrations of poisons. This conclusion could hold good only in cases where the toxic effect was entirely due to the ionized portion of the molecule, otherwise there would be no reason for the expectation of a relation between ionic concentration and toxicity. The author finds that spores are capable of remaining alive a long time, two weeks or more, in the presence of toxic substances, provided the concentration is below that at which plasmolysis occurs. It is also noted that slow growth occurs in toxic solutions slightly below the limit concentrations. As a possible explanation of the mode of action of toxic substances in such cases, the author suggests that the effect of the poisons is due to the hindrance of water-absorption by the protoplasm. This hypothesis, it will be remembered, was suggested long ago by LIVINGSTON,[12] who observed a similarity between the action of salts on the cells of the alga *Stigeoclonium* and the withdrawal of water from the cells. In conclusion, the author points out, with justice, that the composition of the medium should be taken into consideration in studies on toxicity of substances to plants.

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[10] KUNKEL, L. O., The influence of starch, peptone, and sugars on the toxicity of various nitrates to *Monilia sitophila* (Mont.) Sacc. Bull. Torr. Bot. Club 40:625–639. 1913.

———, Physical and chemical factors influencing the toxicity of inorganic salts to *Monilia sitophila* (Mont.) Sacc. *Ibid.* 41:265–293. 1914.

[11] See foregoing review.

[12] LIVINGSTON, B. E., Chemical stimulation of a green alga. Bull. Torr. Bot. Club 32:1–34. 1905.

Le Renard[13] attempts to establish as a measure of the antitoxic action of a substance the "antitoxic coefficient," which is defined as the ratio between the number of liters containing a gram-molecule of the antitoxic substance in centinormal solution (that is, 100 in the case of monovalent substances) and the number of liters in which a gram-molecule of the toxic substance is dissolved at the limit concentration. The propositions which the author derives from the ratio thus defined, and with whose derivation the first part of the paper is concerned, all follow from the arithmetical relations of the quantities involved in the definition, and like the antitoxic coefficient itself have no physiological significance. In his experiments Le Renard studied the antagonistic action with reference to *Penicillium glaucum* of formates, acetates, sulphates, and nitrates of potassium, ammonium, and magnesium, and the phosphates of potassium and ammonium in combination with salts (mostly chlorides and nitrates) of the heavy metals. The antitoxic solutions were used in concentrations ranging in a geometrical progression with a ratio of 1/10, usually from N 10^{-2} to N 10^{-5}. The toxic substance was generally used in one or two arbitrarily chosen concentrations. The results show an unusual regularity for biological data. The chief conclusion of the author is that the resistance of *Penicillium* to poisons varies according to the nutritive medium in one of the following ways: (1) the resistance varies, in a simple ratio, inversely as the concentration of the antitoxic substance; (2) the resistance passes a maximum at a comparatively low degree of concentration of the antitoxic substance; or (3) the resistance is not modified. The first part of this conclusion seems scarcely to be borne out by the data, for in nearly all cases where different concentrations of the toxic substances were used the quantities endured by the fungus fell with a diminution of the concentration of the antitoxic substances. It is probable, furthermore, that the use of a greater number of concentrations with smaller intervals between them would have led the author to modify his conclusion as to the simple relation said to exist between the concentration of an antitoxic substance and its effect on the toxicity of another substance. The recent work of Szücs[14] has clearly shown that the antagonistic ionic effects do not follow any such simple law of proportionality.

The antagonism between the nitrates of calcium, magnesium, and potassium on the one hand, and nitrates of copper, lead, zinc, aluminum, and nickel on the other hand, with reference to the spores of *Glomerella cingulata*, has been investigated by Hawkins.[15] The technique employed was similar to

[13] Le Renard, A., Influence du milieu sur la résistance du Pénicille crustacé aux substances toxiques. Ann. Sci. Nat. Bot. IX. **16**:277–336. 1912.

[14] Szücs, J., Experimentelle Beiträge zu einer Theorie der antagonistischen Ionenwirkungen. Jahrb. Wiss. Bot. **51**:85–142. 1913 (rev. in Bot. Gaz. **56**:85. 1913).

[15] Hawkins, L. A., The influence of calcium, magnesium, and potassium nitrates upon the toxicity of certain heavy metals toward fungus spores. Physiol. Researches **1**:57–92. 1913.

that used and fully described by CLARK. HAWKINS finds in general that the addition of the nitrates of calcium, magnesium, and potassium to solutions of nitrates of copper, lead, or zinc reduces the toxicity of the solutions. With aluminium nitrate no reduction of the toxicity was observed. Nickel nitrate proved toxic only in high concentrations and was not used in combination with the antitoxic nitrates. For combinations of copper nitrate with calcium nitrate it is shown, by considerations based on the mutual influence of salts with a common ion upon the degree of dissociation of each other, and by potentiometer measurements of the concentration of copper ions in the mixture, that neither the reduction of the degree of ionization brought about by the mixing of the salts nor the formation of double salts will account for the lowering of the toxicity of the copper solution by the addition of the calcium salt. For combinations of lead nitrate with nitrates of calcium and magnesium, it is shown that reduction in ionization cannot account for the reduced toxicity of the lead salt. For lead nitrate and zinc nitrate within the limited range of concentrations used (3) there is a constant ratio between the molecular concentration of the toxic salt and that of the antitoxic salt necessary to inhibit the action of increasing concentrations of the toxic salt. For the combination of copper nitrate with calcium nitrate no such constant ratio was evident. The author concludes that the antagonistic action of one salt upon another cannot be attributed, as some investigators have done, either to reduction of the dissociation of the toxic salt or to the formation of undissociated double salts.

Incidental to an investigation, the object of which was to determine the factors inducing the formation of giant cells and mucor yeast by the mucors, RITTER[16] has reported a few experiments on the influence of nitrogenous compounds and sodium chloride on the toxicity of acids. The inhibition of germination of the spores was taken as a criterion of toxicity, although some difficulty was experienced with this test because many of the mucor spores began to form giant cells in concentrations of acid far below the toxic limit. The culture solutions contained, in addition to potassium di-hydrogen phosphate and magnesium sulphate, either peptone or ammonium nitrate and sugar. It was found that malic, citric, tartaric, nitric, and hydrochloric acids were much less toxic in the peptone medium than in the ammonium nitrate medium. The author generalizes from these observations to the effect that the toxicity of organic and inorganic acids is increased by the presence of inorganic nitrogen and depressed by organic nitrogen compounds. The data, it should be remarked, do indeed show an increased toxicity of acids in the presence of ammonium salts as compared with organic nitrogen compounds, but no data showing an absolute increase of toxicity as a result of the addition of ammonium

[16] RITTER, G. C., Die giftige und formative Wirkung der Säuren auf die Mucoraceen und ihre Beziehung zur Mucorhefebildung. Jahrb. Wiss. Bot. 52:351–403. *pl. 1.* 1913.

salts are presented. For combinations of citric acid and sodium chloride it was found that for a wide range of concentrations, mixtures of these substances were more toxic than either alone. In this case the effect seems to be additive. The concentration of hydrogen ions was found to be the chief factor determining the production of giant cells. The production of mucor yeast, which is in no wise related to the production of giant cells, is determined chiefly by the absence of oxygen in slightly acid media containing sugar.—H. HASSELBRING.

Food substances and growth.—The fact that any given result in plant physiology is usually the result of several factors and is only rarely traceable to one factor alone receives further emphasis in the recent work of BOTTOMLY.[17] Mineral nutrients and toxins have received much attention in the discussion of the causes of soil fertility, and both have been shown to be limiting factors in certain cases. BOTTOMLY's work emphasizes the idea that the soluble humus of the soil is an essential factor in soil fertility, providing not only food and energy for numerous soil bacteria, but also serving as a source of food for plants. His interpretation of the work reported in this paper is that the chief interest in it centers around the possibility that the nutrition of a plant depends, not only upon the supply of mineral food constituents, but also upon a supply of certain accessory organic food substances, very small amounts of which are sufficient to supply the needs of the plant. He cites literature indicating that other workers have found that soil humates stimulate the action of nitrogen-fixing bacteria and also that they can be readily assimilated by plants.

BOTTOMLY finds that when peat is submitted to the action of certain aerobic soil organisms (he does not say what ones) at 26° C., it decomposes rapidly "and a large amount of the humic acid present is converted into soluble ammonium humate." His use of the terms "humic acid" and "humates" is interesting in the light of the recent statement by SCHREINER[18] that "the compounds . . . . such as humic acid . . . . have absolutely no existence, but are shown to be mixtures of many widely different compounds." In this connection it may be noted that WIELER[19] has taken the view that "humic acids" in soils are inorganic acids resulting, for example, from the action of bases on salts; and that BAUMANN and GULLY[20] have shown that in peat soils the acid properties are due to the colloidal matter of the cell covering the hyaline sphagnum cells.

BOTTOMLY found that bacterized peat, after sterilization, was an excellent medium in which to grow nitrogen-fixing bacteria and apply them to the soil.

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[17] BOTTOMLY, W. B., The significance of certain food substances for plant growth. Ann. Botany **26**:531–450. 1914.

[18] SCHREINER, O., The organic constituents of soils. Science N.S. **36**:577–587. 1912.

[19] WIELER, A., Pflanzenwachstum und Kalkmangel im Boden. 8vo. pp. vii+235. *figs. 43.* 1912.

[20] BAUMANN and GULLY, quoted in Science N.S. **40**:492. 1914.

A large increase in the nitrogen content of soils resulted from the addition of active bacterized peat as compared with controls of the same soils with sterile peat. It was found that an aqueous extract of bacterized peat supplied all of the plant food necessary for water cultures of tomato, barley, and buckwheat seedlings. It was also found that bacterized peat contains a substance or substances that stimulate growth and enables the plants to utilize the normal mineral food constituents (NH_3, P_2O_5, and K_2O) more readily. It is supposed that in nature these growth-stimulating substances are supplied by the decayed organic matter of the soil. Experiments under way are reported to indicate that during the early stages of the growth of the embryo these substances are supplied from the seed.

The results of these experiments with bacterized peat coordinate well with agricultural practice as observed by the reviewer in the Puget Sound region of the United States. In this region sphagnum bogs are readily converted into productive gardens by drainage and cultivation. This growth-stimulating substance (or substances) is soluble in water and in alcohol and is precipitated by phosphotungstic acid. Very little has been determined as to the nature or composition of these growth-stimulating substances, but they are said to resemble in certain ways the accessory food bodies concerned in animal nutrition—GEORGE B. RIGG.

Some Ontario forest conditions.—In order to obtain some exact data regarding the extent and conditions of their forests, the Commission of Conservation of Canada has had surveyed among other regions a portion of Ontario east of Georgian Bay and north of Lake Ontario. The area is within the basin of the Trent River and comprises some 1,345,000 acres, slightly rolling in character, with a very thin soil over the recently glaciated granitic rocks of Archaean and Ordovician age. HOWE[21] reports that two-thirds of this area was originally covered with a more or less pure white pine forest, the remainder being chiefly of hard wood type, in which beech and maple predominated. Now the virgin forest is practically gone, although on account of the poor quality of the scanty soil less than 12 per cent of the area has been farmed and little more is tillable.

In discussing present conditions, four types of forest are recognized. (1) The pure coniferous forest with less than 10 per cent of other trees is made up of *Pinus Strobus* with a small quantity of *Tsuga canadensis*. It occupies less than 5 per cent of the woodland and is now hardly known in virgin condition. (2) The pure hard wood forest contains less than 10 per cent of coniferous trees and occupies the deeper soils, covering about 33 per cent of the forested area. It is composed of *Acer saccharum*, *Fagus grandifolia*, *Betula lutea*, *Tilia americana*, and a few other minor species. From the predominance of the two species first named, both as mature trees and as seedlings, it is evident that this

[21] HOWE, C. D., and WHITE, J. H., Trent watershed survey. Commission of Conservation, Canada. pp. 156. *ills. 16. maps 3.* 1913.

represents the climax forest of the region. (3) Then comes a mixed forest occupying only some 6 per cent of the wooded area and made up partly of a combination of the previously mentioned types and partly of a swamp type in which *Fraxinus nigra*, *Thuja occidentalis*, and *Abies balsamea* are dominant. (4) Finally, there are areas formerly mostly pine forests, but repeatedly burned after cutting and now occupied by a pioneer association dominated by *Populus tremuloides* and *Betula alba*. It comprises some 56 per cent of the forested area, occupying the thin soils over the granitic or crystalline rocks or the deeper sandy plains and sandy ridges. While potentially pine forest areas, these poplar-birch forests are usually so entirely without pine that only by a system of planting could they be brought to their original richly productive condition.

HOWE reviews at some length the economic loss involved in the forest fires so prevalent in the past and still occurring annually over this region, and shows the true economy of the preventive measures he recommends.

A discussion of the economic and industrial conditions by WHITE and an introduction by FERNOW both show the futility of attempting agriculture in a region so little suited to crop production, and the great importance of having it organized into a forest reserve under government control with scientific supervision.

The illustrations, the excellent index, and the mapping of the distribution of the forest types described all add to the value of the report.—GEO. D. FULLER.

Paleobotanical notes.—SEWARD[22] has published an account of the antarctic ("Terra nova") fossil plants collected by the British Antarctic Expedition of 1910, being the first of the geological memoirs completed. A general account of the various expeditions to this region is given, followed by a description of the paleobotanical material secured, much of it being too fragmentary for certain identification. Among the descriptions are two new genera, obtained from what are probably Mesozoic beds: *Antarcticoxylon* (presumably the stem of a gymnosperm) and *Pityosporites* (thought to be a winged pollen grain of some gymnosperm). Various remains of *Glossopteris* were also identified, and the occurrence of this genus in the antarctic regions suggests a general discussion of the wide uniformity of climatic conditions during the later Paleozoic.

SEWARD,[23] in another paper discussing the Wealden floras, calls attention to the surprising similarity in the general appearance of the floras of Japan, South Africa, North America, South America, Europe, and the Arctic regions. "In the Wealden period the type of vegetation was very similar to that which flourished through the greater part of the world during the whole of the Jurassic,

[22] SEWARD, A. C., Antarctic fossil plants. British Museum, Brit. Antarctic Exped. 1910. Geol. 1:1–49. *pls. 1–8.* 1914.

[23] ———, Wealden floras. Hastings and East Sussex Nat. 2:126–142. *pl. 2.* 1914.

and very shortly after the Wealden the vegetation of the world experienced a very remarkable transformation."

WIELAND[24] has investigated the problematical fossil *Cryptozoon*, and the much discussed question of the origin of the oolites. Oolites and *Cryptozoon* are said to be notable features of the Ozarkian. According to WIELAND, *Cryptozoon* is a marine alga "which formed vast reefs in the Ozarkian oceans"; and in connection with a description of a new species of *Cryptozoon* from Pennsylvania, and the general occurrence of similar forms (as *Eozoon*, for example) in the early Paleozoic, he concludes that the hypothetical "age of seaweeds" preceding the coal plants is a reality.

BERRY[25] has contrasted the ancestry of our present walnuts and hickories, so far as they can be recognized as fossils, back to the Middle Cretaceous, and presents evidence that at this remote period their geographical range and their abundance were much greater than now. This evidence also enables him to explain the geographical distribution of the living representatives of the family.—J. M. C.

Slope-direction and forest distribution.—TURESSON[26] points out that *Pseudotsuga taxifolia* (Douglas spruce or red fir) is confined to north-facing slopes in the Spokane region in eastern Washington. He says "the evidences have shown that exposure is the regulating factor in the distribution of the tree in this region, the northern slopes and ridges being the only localities which offer the needed humidity in soil and atmosphere." He adds "not only around Spokane but in all more or less arid regions can this be observed." He cites from his own observations and from literature several instances illustrating the fact that the southern slope tends to be more xerophytic than the northern. After calling attention to the fact that this tree reaches its best development in the Puget Sound region, he cites COWLES[27] to indicate that near its areal limits a species "can grow only in those formations which resemble most closely in an edaphic way the climatic features at the distribution center." Speaking of the distribution of this tree in the San Juan Islands, he calls attention to the similarity in climate between these islands and the Spokane region. He then says "it is not surprising to find *Pseudotsuga taxifolia* confined to the northern slopes of the hills in these islands." Quoting from a paper by the reviewer,[28]

[24] WIELAND, G. R., Further notes on Ozarkian seaweeds and oolites. Bull. Amer. Mus. Nat. Hist. 33:237–360. *pls. 14–19.* 1914.

[25] BERRY, EDWARD W., Notes on the geological history of the walnuts and hickories. Smithsonian Report for 1913. pp. 319–331. 1914.

[26] TURESSON, G., Slope exposure as a factor in the distribution of *Pseudotsuga taxifolia* in eastern Washington. Bull. Torr. Bot. Club 41:337–345. 1914.

[27] COWLES, H. C., The physiographic ecology of Chicago and vicinity. BOT. GAZ. 31:73–108, 145–182. 1901.

[28] RIGG, G. B., Forest distribution in the San Juan Islands: a preliminary note. Plant World 16:177–182. 1913.

he says, "RIGG has . . . . pointed out the seemingly peculiar distribution of *Pseudotsuga taxifolia* as limited to the north-facing slopes of the hills." It is perhaps generalizing too much to say that the species under discussion is confined to the north-facing slopes "in *all* more or less arid regions," although the phenomenon is undoubtedly of frequent occurrence. If the reviewer interprets COWLES correctly, the absolutely rigid application of the principle quoted from his paper of 1901 is not in accord with the spirit of his more recent teaching. In regard to the quotation from the reviewer's paper, the facts are that the paper cited pointed out *four cases only* in these islands where the forest is largely limited to the north-facing slopes. It would be generalizing too much to say that this is true in all cases in the islands. The whole subject of forest distribution in the San Juan Islands should be made the subject of a careful field investigation. The point of view from which the paper is written is very suggestive and it forms a valuable contribution to the subject of forest distribution in the Northwest.—GEORGE B. RIGG.

Available soil moisture.—ALWAY[29] has grown plants in water tight cylinders until they die from lack of available moisture, and made careful determinations of the moisture conditions of the soils. He concludes that for comparing the available moisture in soils either the wilting coefficient or the hygroscopic coefficient may be used with equal efficiency. The former seems to him preferable in considering conditions of germination and growth of crop plants, and the latter in considering the seed production of such annual plants as field grains and the maintenance of life of perennial plants. Under the conditions of his experiments, most plants seemed capable of producing little or no growth after the soil moisture fell below the wilting coefficient, but whenever there was a well developed root system and no remarkably unfavorable conditions obtained, the plants were able to reduce the moisture content of the soil almost or quite to the hygroscopic coefficient, that is, to 68 per cent of the wilting coefficient. Little difference was found between the ability of the various crop plants used in the experiment to exhaust the soil moisture; while, on the contrary, marked differences were evident in their ability to remain alive after showing injury from drought. Desert legumes of perennial habit remained alive after the water content of the soil had fallen distinctly below the hygroscopic coefficient, showing that the water taken by the soil from a saturated atmosphere may be to some extent available for the maintenance of the life of such plants, although it is evidently beyond the reach of ordinary crop plants. Incidentally, evidence is presented that in many soils of dry lands, the loss of water from all but the thin upper stratum takes places entirely through transpiration.

The bulletin reports a good example of an investigation in a quantitative manner of problems in plant production important alike to agriculture and ecology.—GEO. D. FULLER.

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[29] ALWAY, F. J., Studies on the relation of the non-available water of the soil to the hygroscopic coefficient. Agric. Exp. Sta. Neb. Research Bull. 3:133. *figs. 37*. 1913.

Tree growth.—DOUGLASS[30] hopes to find in tree growth an indicator that may be used for estimating rainfall, but the preliminary steps of his investigation are similar to those of botanical observers. Studying *Pinus ponderosa* grown in the northern plateau of Arizona, a semi-arid region where the amount of precipitation is almost certain to be the limiting factor of annual growth, his conclusions agree with those of KIRKWOOD in indicating the importance of the precipitation of the fall and winter months upon the amount of the increment of the succeeding growing season. Further, a most satisfactory explanation of double annual rings is found in the failure of fall and winter precipitation when the resulting spring drought is followed by the usual heavier rainfall of July and August. Sometimes when this drought is excessive, the later rains do not seem to be able to stimulate a late summer growth, and a very narrow single ring results. In a few instances DOUGLASS thinks that, for some unknown reason, there has been the entire suppression of one annual ring.

From measurements of annual rings, given in detail in a previous paper,[31] a growth record is obtained for the past five centuries. When this is plotted as a curve and a comparison made between an available rainfall record for the region extending back to 1867 and the portion of the growth curve for the corresponding period, there is found an agreement of 80 per cent, but we are warned that such an agreement is likely to obtain only for a dry climate. An effort to discover a regular periodicity in the growth rate is rather unsuccessful, although there seems to be some agreement with the sun-spot cycle of 11.4 years.—GEO. D. FULLER.

A hydrarch succession.—MATTHEWS[32] has reported on the study of the succession of plant associations occurring in the gradual filling up of a pond of some 16 acres in area situated in Perthshire, Scotland. Aquatic and marsh associations of the usual type are found, but with an unusual paucity of species. —GEO. D. FULLER.

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[30] DOUGLASS, A. E., A method of estimating rainfall by the growth of trees. Bull. Amer. Geogr. Soc. 46:321–335. 1914.

[31] ——— Weather cycles in the growth of big trees. Month. Weather Rev. 37:225–237. 1909.

[32] MATTHEWS, J. R., The White Moss Loch: a study in biotic succession. New Phytol. 13:134–148. *figs. 2.* 1914.

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A STUDY OF DELAYED GERMINATION IN ECONOMIC SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 204

DEAN H. ROSE

(WITH ONE FIGURE)

This paper presents the results of an attempt to discover some of the practical problems that seedsmen and growers have to meet, and to work out, so far as possible, practical methods of solving these problems. The seeds tested were furnished by six of the leading seed houses of the United States.

In the present state of our knowledge it can be said that delayed germination and poor germination are due to one or more of the following causes: hard-coatedness, the need of after-ripening, exclusion of oxygen by the seed coat, the effect of frost on seeds, fungi on or in seeds, and of course the presence of seeds containing dead embryos.

Hard-coatedness

The condition of hard-coatedness in the seeds of legumes is well known. To overcome this condition investigators have used hot water, chemicals, and mechanical devices for scratching or puncturing the seed coat.

The use of hot water for forcing germination is undoubtedly older than the references to it in periodical literature. It was recommended by BRUYNING (6) in 1893 for seeds of *Ulex europaeus*,

and by WERNICKE (30) in 1895 for several different kinds of seeds. Mention may also be made of the work of JARZYMOWSKI (17) in 1905 with seeds of various economic legumes, and of BOLLEY (3) in 1912 with those of alfalfa. BOLLEY obtained positive improvement in germination if exposure to a given temperature was not long enough to kill the embryo.

Treatment with concentrated sulphuric acid dates from the work of ROSTRUP (25) in 1896–1897. It was also used by TODARO (29) in 1901, by HILTNER (14) in 1902, by JARZYMOWSKI (17) in 1905, and by BOLLEY (3), and LOVE and LEIGHTY (22) eight years later. Increased germination was obtained in all these cases.

Treatment with other chemicals has included the use of ether, ethyl, and other alcohols (VERSCHAFFELT 31, 1912), chloroform, sodium hydroxide, postassium hydroxide, potassium nitrate, and mercuric chloride. Of these, the lower alcohols are the only ones that are very effective.

There are obvious practical objections, however, to the use of either hot water or chemicals. As a consequence, there have been numerous efforts to devise means for the mechanical treatment of hard-coated seeds. In Germany, KUNTZE and HUSS (16), working about 1890, were able with a scratching machine to increase the germination of *Lathyrus sylvestris* 83 per cent, *Vicia Cracca* 71 per cent, and *Astragallus Glycyphyllos* 77 per cent.

Somewhat later MICHALOWSKI devised an apparatus in which the seeds were passed between two rollers, one of rubber, the other of rough steel. Smaller sorts of seeds were badly crushed by such a mechanism, and it was later displaced by two others, one designed by the Wissinger Seed Co., of Berlin, the other, called a "preparator," by H. NILSSON of the experiment station at Svalöf, Sweden. In both of these the seeds are thrown from a revolving disk against the concave surface of a circular rough stone, within which the disk revolves. HUME and GARVER (15), using the "preparator," obtained a definite increase in the germination of seeds of *Medicago sativa*, *M. media*, and *M. falcata*. Another machine now in use in England has made it possible, according to CARRUTHERS (7), the designer, to buy clover seed guaranteed to germinate 98–100 per cent. The seeds to be treated are fed into a revolving cylinder

lined with sharp, close-set steel points against which the seeds are thrown and scratched as the cylinder revolves.

Mention should be made here also of an apparatus invented by KÜHLE (19) for scraping the rough outer covering from sugar beet "seed." Very satisfactory results have been obtained by its use, since "seeds" so treated absorb water better than untreated ones, and germinate more rapidly; they also give a better total germination, on account of the removal of fungus-infected material from the outside of the "seed," especially if this removal is followed by treatment with some fungicide.

With any one of the machines here described except the last, which serves a slightly different purpose, it has been found difficult to treat every seed that passes through and, at the same time, to avoid serious cracking of the coat or bruising of the entire seed (GLOCKENTOEGER 11).

It is believed that these difficulties have been avoided in a machine devised and in use during the winter of 1912–1913 at the Hull Botanical Laboratory of the University of Chicago. This machine consists of a direct pressure blower, furnished by the Connersville Blower Co., to which is attached an apparatus through which seeds can be fed and blown against the points of a bank of needles. In experiments conducted with this machine, the blower was driven by a two horse-power motor and gave pressures as high as 2.5 pounds to the square inch. The needles used were of three sizes, nos. 4 and 11 sewing needles and no. 4 darning needles, made up into three different cylindrical bunches or banks, each bank of course consisting of only one size of needles. The needles were held together by solder at the eye end and by wire or a ferrule one-half to two-thirds of the distance from the eye to the point.

In the cut here shown (fig. 1) the needles are about half an inch from the end of the air tube. In practice a screen cap is placed over the needles and the tube as a covering for a glass jar beneath, into which the seeds fall. To use the apparatus, valve *e* is closed and valve *b* is opened; seeds are poured into compartment *c;* valve *b* is closed and the blower started; valve *e* is then opened wide enough to let the seeds out, but not so wide that they interfere with each other as they strike the needle points. It is

plain that the distance the valve is to be opened will vary with different seeds, but will not be at any time particularly hard to determine. With valve *b* closed, there was no difficulty in getting the seeds down into the air tube; with it open they would be blown out at *d*.

The opening at *h* is five-eighths of an inch in diameter and will accommodate seeds of sweet peas, lupines, *Lathyrus*, honey locust,

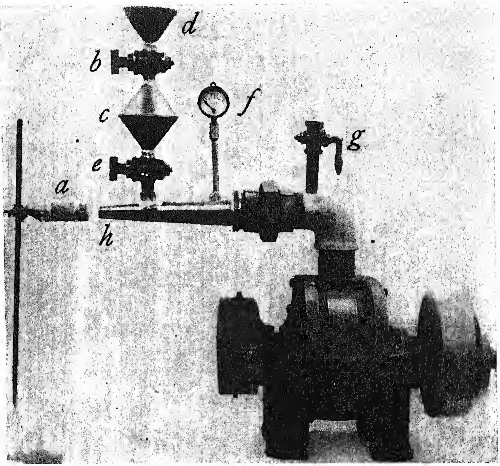

FIG. 1.—Apparatus for increasing the germination of hard-coated seeds by blowing them against a bank of needle points.

etc. When smaller seeds are to be treated, an attachment with three-eighths inch feed, valves, and air tube can be adjusted readily. Valve *g* can be used to regulate the pressure, as read on the pressure gauge *f*.

Tests were made of treated and untreated seeds, on filter paper kept moist with distilled water, at a temperature of 23–25° C. The results are summarized in table I.

It is plain that treatment with the machine increased germination considerably in the case not only of legumes, but also of snapdragon, *Delphinium*, sweet marjoram, *Ipomoea*, okra, and lettuce.

TABLE I

GERMINATION OF SEED TREATED WITH MACHINE

Kind of seed	Duration of test in days	Germination	
		Untreated	Treated
Alfalfa	10	51	99
Alfalfa	10	74	94
Bossiaea heterophylla	30	3	73
Bossiaea scolopendria	30	11	52
Clianthus Dampieri	10	0	80
White clover	10	70	94
Delphinium chinense	30	74	96
Dilliwynia ericifolia	30	2	77
Gleditschia	90	0	70
Ipomoea (average of 3 spp.)	10	34	71
Lathyrus (average of 3 vars.)	14	63	93
Lettuce (average of 11 vars.)	12	55	83
Sweet marjoram	30	54	96
Mustard (average of 2 vars.)	14	20	69
Okra	30	34	79
Perennial peas (average of 2 vars.)	10	62	87
Sweet peas (average of 5 vars.)	14	70	99
Platylobium trilobatum	30	13	64
Snapdragon	30	6	23
Vetch	10	77	92
Average		40.7	83.7
Increase due to treatment			43.7

It is not strictly correct, however, to call lettuce seeds hard-coated. That their germination is improved by treatment with the machine shows delay to be due to coat restrictions. The coat restrictions are removed also by soaking in water for 24 hours, hence it is likely that delay is caused by a reduction in rate of water absorption rather than by lack of oxygen. Whatever be the character of the seed coat, or coats, which interferes with germination, it disappears as the seed grows older. These points are well illustrated by table II. The data here presented support the statement made by various seedsmen, that two or three-year-old lettuce seed gives better germination than fresh. How this condition comes about is not known, but it probably depends on

changes in the permeability of the seed coat. It is certainly not a matter of embryo changes.

TABLE II

GERMINATION OF TREATED AND UNTREATED LETTUCE SEED

VARIETY	DURATION OF TEST IN DAYS	GERMINATION		
		Untreated	Treated with machine	Soaked in H_2O 24 hrs.
Black-seeded butter—				
1909	12	98	....	....
1910	12	99	....	....
1911	12	89	93	95
1912	12	67	97	100
Prize head—				
1909	12	98	....	....
1910	12	97	....	....
1911	12	84	96	97
1912	12	76	98	96

It appears, from a study of the records kept in this work, that not only the total germination, but also the rapidity or energy of germination is greater in treated than in untreated seeds. This is shown in table III.

TABLE III

RAPIDITY OF GERMINATION OF TREATED AND UNTREATED SEEDS*

Kind of seed	Germination after	Untreated	Treated
Alfalfa	3 days	48	98
White clover	3 "	69	89
Perennial peas	4 "	0	33
Perennial peas	4 "	24	80
Lupines	4 "	40	88
Delphinium	5 "	4	44
Sweet peas	4 "	0	70
Sweet peas	4 "	16	86

*For final germination percentage of these samples see table I.

Such rapidity of germination would clearly be of advantage in making the crop uniform in size and age, and in keeping down weeds. Indeed, it may be said truly that vigor of germination and vigor of the plants produced are more important than merely high germination percentage. Plants that get a good strong start

are more certain to be productive than those that for any reason are weak from the beginning.

Before this machine can become commercially practicable, experiments must be conducted to determine: (1) the possibility of substituting something else for needle points; (2) the proper distance these points should be from each other to give the best results for different sized seeds; (3) the pressure necessary to give the best germination for different kinds of seeds; for certain legumes a pressure of two to three pounds is necessary, for lettuce one pound or even less; (4) the effect of storage on the germination of treated seeds; (5) the germination of treated seeds in soil. To be effective in overcoming hard-coatedness, the needle point need only pass through the palisade layer and not entirely through the coat. Even with this slight deformation it is possible that bacteria and fungi can gain an entrance. That destruction by bacteria and fungi actually does take place was shown by JARZYMOWSKI (17) for seeds of *Ulex europaea*, lupines, and other large-seeded legumes which had been treated with the Wissinger machine. Red clover and *Lotus corniculatus* were the only ones whose germination in soil after treatment was not seriously reduced.

As to the germination in soil of seeds treated by the blowing method here described, there are not at present enough data on hand to justify the drawing of definite conclusions. Preliminary experiments seem to indicate for alfalfa seed, where the percentage of hard seed is high, that germination in soil is definitely better after treatment than before. Further investigation, of course, is necessary before this can be confirmed. In conclusion it may be stated that there was no serious crushing or cracking of seeds or seed coats by this machine.

The need of after-ripening

This is a condition which occurs, to mention only a few cases, in seeds of *Crataegus*, various conifers, *Fraxinus*, potato tubers, and lily-of-the-valley bulbs. For a discussion of the general situation and a résumé of the literature the reader is referred to the paper by ECKERSON (10) dealing with after-ripening in the seeds of *Crataegus*. The work to be discussed here had to do with the

germination of seeds of conifers, and specific reference will be made to a few of the more pertinent papers on the subject.

It is a matter of common knowledge that conifer seeds germinate slowly. It is also well known for several of them that as they grow older the rapidity of germination increases, up to at least the end of the first 6 months after they were gathered. SCHWAPPACH (27) states that in the fall seeds of *Abies* did not begin to show sprouts for 60 days, and required 40 days more before the test could be considered closed. In March they began almost immediately and finished in 20 days. The conclusion is natural that after-ripening takes place, and this, in fact, is assumed by workers who have recently attacked the problem. HILTNER and KINZEL (14), it is true, reasoning from results obtained by treating seeds of *Pinus Strobus*, *P. Peuce*, and *P. Cembra* with concentrated sulphuric acid, ascribed the delay to coat restrictions. LAKON (20) has made the objection that the tests on which these authors rely were too few and on too small a number of seeds. He repeated their experiments with the same three species of pine, but could obtain no forcing of germination. Untreated seeds took up water just as well as did the treated ones, even though their outer coats were hard. Careful determinations of the amount of water absorbed by untreated seeds of *Pinus sylvestris*, *P. Strobus*, *P. Peuce*, and *P. Cembra* showed that all of them reached nearly the maximum in 24–48 hours. Increases in weight after that time were practically negligible; hence it is clear that such seeds cannot be considered "hard-coated" like the seeds of legumes. Moreover, the cutting test, applied to these seeds, showed that all of them were damp, that is, had absorbed water. Increases in weight, therefore, were not due to a few easily swelling seeds. From these results LAKON concludes that conifer seeds are not, strictly speaking, "hard-coated," and that delay is due to conditions within the embryo.

Although LAKON found concentrated sulphuric acid ineffective, CORREVON states, in a paper published somewhat earlier, that weak acid (0.25 per cent acetic or 2 per cent phosphoric) increases the germination of seeds of *Juniperus Cedrus*.

SCHWAPPACH recommends cold storage for 14–30 days (he does not say how cold) for seeds of *Pinus Strobus*, followed by a germi-

nation temperature of 25° C. The common practice of layering various conifer seeds doubtless finds its justification in a shortening of the time required for germination. Low temperatures have also been used for preserving the vitality of conifer seeds. HAACK (12) dried seeds so they lost about 2 per cent in weight, then stored them in dry, air-tight containers on ice. CLEMENS (9) stored seeds in the refrigerator of a brewery in vessels containing sodium carbonate to absorb moisture and carbonic acid. Both investigators report that seeds thus stored remained viable longer than those kept under ordinary laboratory conditions.

In order to analyze the situation more carefully, the following series of experiments were conducted on seeds of various conifers.

1. Tests of untreated seeds.

2. Tests of seeds which had been in cold storage (3–5° C.): (*a*) in wet sand, (*b*) in weak solutions of hydrochloric acid, (*c*) in distilled water.

3. Tests were made with seeds which had been injected with weak hydrochloric acid or with water, by exhausting the air from them when they were in these liquids, and then restoring the pressure to normal. This was repeated at least three times for all seeds here spoken of as injected. Table IV summarizes the results obtained in series 1 and 2*a*.

TABLE IV

GERMINATION OF CONIFER SEEDS, UNTREATED AND AFTER STORAGE IN WET SAND AT 3–5° C.; PERCENTAGE GERMINATING AFTER ONE MONTH IN GERMINATOR

KIND OF SEED	DRY STORAGE UNTREATED				COLD WET STORAGE FOR			
					1 month	2 months	3 months	4 months
	Date of starting germination							
	Jan. 26	April 4	April 24	May 30	Feb. 26	Mar. 25	April 26	May 28
Cupressus macrocarpa ...	8	0	1	......	15	27	40	36
Picea Menziesii.........	56	42	......	23	70	60	......	......
Pinus austriaca.........	57	48	27	28	78	88	......	64
Pinus Strobus..........	12	10	6	28	34	40	44	59

The results given in the table show that germination was definitely increased by cold wet storage for four kinds of conifers.

In no case does the maximum germination of seeds of a given kind from dry storage equal that of seeds of the same kind from cold wet storage. The effect of the cold wet storage is most noticeable in the cases of *Pinus Strobus* and *Cupressus macrocarpa*, where increases of 32 per cent and 31 per cent respectively were obtained. For all of the seeds here reported on, except those of *Pinus Strobus*, dry storage seemed to cause a decrease in viability. This can be seen from the first four columns of the table. Tests of *Pinus Strobus* were run for 60 days, the other three for 30 days.

TABLE V

GERMINATION OF SEEDS OF CONIFERS AFTER SOAKING IN WEAK ACID AND STORAGE AT 3–5° C.

KIND OF SEED AND LENGTH OF TIME IN COLD STORAGE	CON-TROL	ACID (HCl)					WATER	
		Soaked			Injected		Soaked	Injected
		n/20,000	*n*/10,000	*n*/6,400	*n*/20,000	*n*/6,400		
Pinus Strobus—								
3 days	......	44	58	53	66	54	......	......
6 "	......	51	57	44	......	......	......	......
10 "	......	53	65	67	......	......	63	......
No cold storage	30	......	......	......	......	......	50	68
Pinus austriaca—								
3 days	......	64	......	52	......	......	65	......
6 "	......	48	......	42	......	......	59	......
10 "	......	35	......	50	......	......	45	......
No cold storage	52	54	......	......	......	......	......	......

The series of experiments shown in Table V was planned to determine whether delay in germination is due to an alkaline or neutral reaction of the embryo. It was thought that if such is the case, weak acid solutions would change the reaction sufficiently to cause growth to begin, when the seeds were placed in the proper conditions.

The results obtained for *Pinus Strobus* do not, however, bear out this theory. Seeds injected with distilled water gave 18 per cent better germination than those merely soaked in it, and slightly better than those injected with weak hydrochloric acid. It seems likely from this that delay is due merely to lack of water. When

this water was supplied, by long soaking or by forcible injection under pressure, germination was much improved.

Results for *Pinus austriaca* are less conclusive on this point. They do show, however, and the same is true for *P. Strobus*, that soaking in either water or weak acid gave greater germination than was obtained in the controls, 38 per cent and 13 per cent respectively. For *P. austriaca*, better results were obtained from short than from long soaking.

Referring again to table IV, it is possible that the increases in germination shown there were due not so much to the cold storage in itself as to the thorough infiltration of the seeds with water. There is need of much more work on this question before any definite conclusions can be drawn.

Exclusion of oxygen

No attempt will be made to review former work, since this has already been done by SHULL (28). Results presented in table VI seem to indicate that the germination of certain economic seeds is delayed for lack of oxygen. They also indicate the need of a detailed study of these seeds.

TABLE VI

GERMINATION OF SEEDS TREATED WITH OXYGEN OR HYDROGEN PEROXIDE

Kind of seed	Duration of test in days	Untreated	In 80 per cent oxygen	In 0.15 per cent H_2O_2
Dandelion	14	56	....	72
Datura—				
Golden Queen	14	73	....	70
Wrightii	14	20	....	100
Lettuce—				
Grand Rapids	10	0	44	32
Martynia	20	0	90	80

Datura Wrightii was forced considerably by hydrogen peroxide, *Datura* Golden Queen not at all. Lettuce gave good results, but, as has been suggested, this is probably due to absorption of water.

Effect of frost on seeds

ATTERBERG (2) says that seeds of oats and barley harvested in Sweden after a heavy frost gave fair to good germination in the

laboratory, but in many cases no plants when sown in the field. Unpublished data obtained by EASTHAM in Canada show that the germination of oats grown in the prairie provinces is often seriously reduced by early frosts. He says, "as far as our observations go, a couple of degrees of frost in the milk stage are in many instances sufficient to ruin oats for seed. In the dough stage they are not nearly so susceptible, and when well ripened and dry stand considerable frost without serious injury." EASTHAM found also that such seed, germinating poorly when harvested, often improved with age. This seems to indicate in such cases the necessity for a period of after-ripening. Through the courtesy of the Canadian seed laboratory and two American seed houses the author has had the privilege of testing several samples of frosted oats. The results are summarized in table VII.

TABLE VII

GERMINATION OF OATS

Variety	Untreated; on filter paper at 20° C.	Untreated; sender's test 18°–20° C.	Hulls off; on filter paper at 20° C.	Hulls removed; on cotton in petri dishes at 22° C.			
				80 per cent oxygen	60 per cent oxygen	40 per cent oxygen	20 per cent oxygen
Lincoln	85	40	79	....	....	....	....
Swedish	77	40	80	....	....	....	....
4920	34	35	38	31	25	38	33
5139	46	....	....	....	....	....	....
3477	56	43	....	....	....	....	....
3974	45	42	....	....	....	....	....
4948	89	62	94	93	97	95	96
5302	16	27	....	....	....	....	....

The best germination was obtained from hulled seeds in oxygen, though the results are more clear-cut for no. 4948 than for no. 4920. No definite conclusions can be drawn as to what percentage of oxygen is most effective. It is noteworthy that two samples, nos. 3477 and 4948, show much better germination, 23 and 27 per cent respectively, than they did when tested in the Canadian Seed Laboratory six months earlier. This agrees well with the statement made above that frosted oats go through a process of after-ripening and improve in viability as they grow older. There is the same need of after-ripening in wild oats (*Avena fatua*), as has been

shown by Atwood. But referring to the table again, it will be seen that one sample, no. 5302, deteriorated in vigor as it grew older, since it gave a percentage of 27 when first tested and 11 per cent less 6 months afterward. Considering the results as a whole, it is clear that frosted oats are unreliable in performance and of very doubtful value for seeding purposes.

Another crop which sometimes suffers from frost is garden peas. Within the last two or three years the growing of garden peas for seed has become an important industry in Idaho and Montana. It has been found that certain of the late varieties grown there are injured by frost and the viability of the seed seriously impaired.

A study of 14 samples of such peas has shown that decrease in germination is probably due to two different causes, both of which, however, may be the effect of frost.

1. Actual injury to the embryo, especially the tip of the radicle. It has a whitish shriveled appearance and starts to grow very slowly, if at all.

TABLE VIII

Germination of peas

Variety	Coats on; 20°	Coats on; 25°	Coats off; 20°
Premium Gem	90	82	86
Nott's Excelsior 73186	68	....	....
" " 71531	98	100	....
Telephone 885H	58	46	55
Gradus 913K	44	36	58
" 913S	70	....	76
Dwarf Defiance 874C	56	....	52
" " 874K	64	....	96
" " 874H	78	....	76
Alderman 912S	66	....	....
" 912K	82	....	96
" 912T	56	....	....
" 912V	78	....	92
Telegraph 68528	48	....	50
Average	67	....	74

2. The presence of fungi on or in the seed coat. That this actually decreases germination was shown by the work summarized in table VIII. With the coats on, the seeds of all the samples here reported on showed much fungus infection; with coats off,

very little or even none at all. Ten samples gave an average germination of 67 per cent with the coats on, and 74 per cent with the coats off, a difference of 7 per cent. Individual samples, such as Dwarf Defiance 874K, gave even more striking results.

The condition of garden peas, with reference to fungi, is approached more or less closely by that of a large number of other garden and flower seeds as is shown in table IX, summarizing the general results of this investigation, and in the discussion following.

Plants whose seeds show delayed germination, classified according to probable causes (the word "probable" is used advisedly, for while the evidence is convincing in some cases, it is much less so in others):

1. Hard-coatedness.—*Canna*, *Clianthus Dampieri*, *Delphinium*, *Erythrina*, *Hibiscus*, *Ipomoea* (4 spp.), *Lathryus*, *Lupinus*, sweet peas (4 vars.), snapdragon, alfalfa, sweet clover, white clover, lettuce (10 vars.), mustard (2 vars.), okra, sensitive plant, sweet marjoram, vetch, *Gleditschia*.

2. Frosted.—Oats, peas (8 vars.).

3. Need of after-ripening.—Wild cucumber, *Picea* (3 spp.), *Pinus* (2 spp.).

4. Exclusion of oxygen by the seed coat.—*Datura Wrightii*, *Martynia*.

5. Cause of delay not determined.—*Coix Lachryma*, feather grass, Pampas grass, asparagus, barley, blue grass, cardoon, celery, chives, dill, horehound, kaffir corn, leek, millet, parsley, parsnip, pepper, radish, rosemary, spinach, summer savory, thyme, *Aquilegia*, *Asparagus Sprengeri*, *Bignonia*, *Centaurea*, *Clematis*, dandelion, *Datura* Golden Queen, *Eschscholzia*, foxglove, heliotrope, *Helianthus*, hop, lavender, *Momordica*, *Nasturtium*, *Oenothera*, pansy, *Pentstemon*, *Primula*, *Salvia*, *Verbena*, *Abies Mertensiana*, *A. pectinata*, *Berberis*, *Betula alba*, *Cupressus horizontalis*, *C. macrocarpa*, *C. pyramidalis*, *Larix*. Further work would doubtless explain the cause of delay in many of these seeds and make the growing of plants from them a much simpler matter than it now is.

Very pertinent at this point are the results from the Minnesota Seed Laboratory for 1910 and 1911 (OSWALD 23). Of field seeds 14 kinds were tested; of garden seeds 26 kinds. Of field seeds, for

TABLE IX

SHOWING THE NUMBER OF SPECIES AND VARIETIES TESTED, CLASSIFIED ACCORDING TO THE QUALITY OF GERMINATION AND THE PROBABLE CAUSES OF DELAY

SPECIES AND VARIETIES	GERMINATION				PROBABLE CAUSE OF DELAY						INFECTED WITH FUNGI
	Good	Poor or slow	None	Totals	Hard-coated-ness	Frost injury	Exclusion of oxygen by seed coat	Need of after-ripening	Not de-termined	Totals	
Ornamental grasses	......	......	3	3	......	......	......	......	3	3	......
Field and garden plants	22	44	2	68	16	9	......	......	19	44	31
Ornamental flowering plants	11	35	3	49	19	......	2	1	21	43	26
Shrubs and trees	......	9	5	14	1	......	......	5	8	14	12
Totals	33	88	13	134	36	9	2	6	51	104	69

3 of the kinds, 50 per cent or more of the samples were below the government standard of germination. Of garden seeds, for 16 of the 26 kinds, 50 per cent or more of the samples were below standard. Mention should be made also of work by BROWN (5) on the germination of packeted vegetable seeds. He found that the average germination of box vegetable seeds put up by 60 firms for four years was 60.5 per cent. The lowest average for any firm was 36.5 per cent, the highest 81.5 per cent. The average germination of packeted vegetable seeds put up by 20 mail-order houses in 1911 was 77.5 per cent (lowest average 76.2 per cent, highest 77.5 per cent). Just what these figures signify is not clear. There are three possibilities: (1) the seeds were poor because of the seedsman's dishonesty or carelessness; (2) the seeds were poor because it is not possible with present methods to produce better ones; if so, the government standard is, now at least, too high and methods of production need improvement; (3) the seeds seemed poor because present methods of making germination tests do not always adequately determine the value of a given sample. In the writer's opinion, the responsibility for low test must be shared about equally by all three, though the first is a less important factor than it was a few years ago.

6. Plants whose seeds were found infected with fungi.—Feather grass, asparagus, beggar weed, buckwheat, cardoon, celery, chives, sweet clover, dill, kaffir corn, leek, millet, oats, parsley, peas (12 vars.), pepper, radish, rosemary, spinach, thyme, vetch, *Aquilegia*, *Asparagus Sprengeri*, *Bignonia*, *Clematis*, *Clianthus Dampieri*, wild cucumber, dandelion, *Datura* Golden Queen, *D. Wrightii*, *Helianthus*, hop, *Ipomoea* (4 spp.), *Lathyrus*, lavender, *Nasturtium*, pansy, sweet peas (4 vars.), *Primula*, *Verbena*, *Abies Mertensiana*, *A. pectinata*, *Berberis*, *Cupressus horizontalis*, *C. macrocarpa*, *C. pyramidalis*, *Picea excelsa*, *P. Menziesii*, *P. rubra*, *Pinus austriaca*, *P. Strobus*.

The species and varieties tested were 134, but 30 of these are omitted from the second section of table IX; 29 of these germinated rapidly and well, and one other, on account of bad infection with fungi, showed not delayed but definitely poor germination; 69, or 51.4 per cent of the total, were found more or less infected with

fungi. This point was determined for each kind of seed not more than two days after the test began. All filter paper was boiled 5–10 minutes before being used and kept moist with distilled water during the test. Repeated washing of seeds and removal to fresh filter paper showed that in all cases infection came from the seeds, not from the paper.

There is no intention here of implying that seedsmen in general purposely put on the market seeds low in vitality or badly infected with fungi. It does seem clear, however, that there is need of closer supervision by the seedsmen themselves of all stages of the process of seed production; alternation of crops to avoid soil-infection, cultivation, harvesting, threshing, cleaning, storage; all of these need close attention if seed of the best quality is to be produced. The most candid way in which to approach the whole question is to admit that seed analysts, seed-growers, and seed merchants do not at present know a number of things they need to know in reference to the question of fungus infection of seeds, and to all the other questions considered in this paper. The whole matter constitutes an extremely complex physiological and pathological problem, with very practical aspects, the solution of which can be brought about only by careful study from several different points of view. To be specific, the following lines for investigation may be suggested:

1. The relation of germinator tests to the actual vegetation of seeds in the soil. This should be studied through a period of several years.

2. The relation of fungi on or inside of seeds to the germination of such seeds in soil. At the risk of seeming to repeat unnecessarily, the writer wishes to say that in his opinion the importance of this problem is only poorly appreciated in this country. Some recognition of the dangers accompanying fungus infection of seed has appeared of late in the work of BOLLEY and others in the United States, and in German agricultural literature. APPEL (1), writing on the relation of pathology to seed control, says that in seed-testing stations, pains should be taken to give judgment as to the presence of spores of plant diseases on seeds to be examined. It is his opinion, further, that in comparative field tests more

attention must be given than formerly to pathological phenomena. Observations on this point should be given along with other data from the experiment.

3. The causes of delayed germination in asters, certain hardy perennials, labiates, ornamental grasses, cucurbits, conifers, frosted oats, *Betula*, and *Berberis*.

4. The value of hard seeds of legumes when planted in the soil.

5. The relation of any or all of the causes of delayed germination to the vigor of the plants produced. It is not enough that a given lot of seeds shall be free from impurities; it is not even enough that it shall give a high germination percentage. It must, above all, give rise to vigorous productive plants, when planted in field conditions. Consequently, any knowledge which will teach us how to grow such seeds and how to know poor seeds is of the greatest practical importance.

Summary

1. Hard-coated seeds of legumes, and seeds of *Delphinium*, *Ipomoea*, lettuce, mustard, okra, sweet marjoram, and snapdragon can be forced to more rapid germination by being blown against needle points.

2. For two varieties of lettuce it is shown that the seed improves in viability as it grows older, up to the end of at least the fourth year. This improvement is probably due to increased permeability of the inner seed coat to water.

3. Cold storage in wet sand increased the germination seeds of *Pinus Strobus* by 32 per cent, of *Cupressus macrocarpa* by 31 per cent. Delayed germination of conifer seeds, more especially those of *Pinus Strobus* and *P. austriaca*, seems to be due to lack of water intake, and not to an alkaline or neutral reaction of the embryo. This statement is supported by the fact that seeds injected with distilled water gave better germination than those merely soaked in water or in weak acid at the temperature of melting ice. Any kind of soaking or injection gave 13–38 per cent better germination than was obtained with the controls.

4. Certain samples of frosted oats improve in germinating power as they grow older, others deteriorate.

5. Certain late varieties of western-grown garden peas germinate poorly. This is shown to be due to one or both of two causes: (*a*) actual frost injury to the embryo; (*b*) the presence of fungi on or in the seed coat or inside of it.

6. Seeds of 51.4 per cent of all species and varieties examined showed fungi on the seed coat within two days after being put to germinate.

The writer is indebted to Dr. WILLIAM CROCKER for many suggestions and criticisms.

UNIVERSITY OF CHICAGO

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SPECIFIC ACTION OF ORGANIC COMPOUNDS IN MODIFYING PLANT CHARACTERISTICS; METHYL GLYCOCOLL VERSUS GLYCOCOLL

OSWALD SCHREINER AND J. J. SKINNER

(WITH FOUR FIGURES)

The effect on plant growth of a large number of soil organic compounds and other organic substances has been tested in this laboratory from time to time. The action of the two compounds glycocoll and methyl glycocoll on plants is very interesting, the former being beneficial, while the latter is harmful to growth and affects the plants in a peculiar way.

The utilization of certain nitrogenous compounds by plants, some having a beneficial effect and replacing nitrates in their action, and others having harmful effects, producing peculiar characteristics, leaves but little doubt that organic compounds in soils or nutrient solutions are absorbed directly by the roots of plants and enter into the cells, reacting with the cell contents and producing effects which differ according to the nature of the compound absorbed. The process is connected with and is a part of the general metabolic processes of plants. The absorbed material passes through the membranes possessing these properties of absorption, and reacts on the cell contents in a favorable or unfavorable manner, influencing the life processes of the plant itself. Glycocoll, a nitrogenous compound having a definite chemical structure, is shown to have been absorbed or used by the plant to build up its tissue, while the related compound, methyl glycocoll, also nitrogenous but having a different chemical structure, is absorbed by the plants and has an unfavorable influence, causing decreased growth, and a peculiar twisted lateral growth of the leaf of the plant.

The properties of plants of absorbing the mineral constituents from the nutrient or soil solution do not differ in respect to the absorption of the organic constituents from the solution. In the case of the methyl glycocoll the greatest harmful effect was noted

in those solutions where there was the greatest absorption of inorganic nutrients, and while no methods are available to study the absorption of this organic compound, it seems justifiable to assume that the cultures also absorbed a greater amount of organic constituent from the solution, while in other solutions where the absorption of nutrients was small the harmfulness of the methyl glycocoll was also only slight, indicating that a small amount was absorbed.

It has been shown in this laboratory and elsewhere that plant roots can affect organic substances externally, and it is therefore possible that organic substances may also influence the plant itself through this external action; however, in the majority of cases that have come under our observation the compounds have been absorbed, for in such cases as could be tested they disappeared from the solution and had their effects on the plant tops either favorably or unfavorably, as the case may be.

In the case of dihydroxystearic acid the normal metabolism is greatly disturbed.[1] This is shown by the difference in the absorption of the separate nutrients, phosphate, nitrate, and potash, a proportionately greater nitrate consumption being evident with the plants affected by dihydroxystearic acid. Such a change in metabolism could be explained only on the assumption that the compound produced a reaction within the plant after absorption from the solution.

The specific effects produced by organic compounds must also be taken into consideration. For instance, cumarin produces greatly stunted tops, with short, broad leaves and much distorted and thickened stems in the case of wheat plants. Quinone, on the other hand, produces long, slender, thin plants. The different reactions of the plants to these two compounds must be due to the direct absorption of the compound accompanied by a disturbed metabolism. Moreover, the cumarin-affected plants absorb relatively more phosphate, the quinone plants relatively more potash.[2]

Furthermore, plants grown in guanidine solutions develop small spots of a bleached appearance which grow and spread, producing

[1] SCHREINER, O., and SKINNER, J. J., Some effects of a harmful organic soil constituent. BOT. GAZ. 50:161. 1910.

[2] ———, The toxic action of organic compounds as modified by fertilizer salts. BOT. GAZ. 54:31. 1912.

a weakened plant, the leaves of which break at the stem, wilt, and die, the roots remaining unaffected.[3] This is explainable only on the assumption of its absorption by the plant and its action on the plant protoplasm.

The cases cited of the beneficial and harmful action of organic compounds, many having specific characteristics, are sufficient to show that organic substances, like inorganic poisons, are absorbed and react with the protoplasm of the plant.

The effect of methyl glycocoll on plants has not previously been determined. In the early experiments[4] of this laboratory glycocoll was shown to be beneficial to wheat seedlings in distilled water cultures. Concentrations of 1 to 1000 parts per million were used. The tops were increased in all the cultures; the roots were slightly injured by the higher amounts.

HANSTEEN[5] showed that glycocoll was slightly harmful to *Lemna*, and that from it were produced proteins. In MOLLIARD'S[6] experiments beneficial results were secured in water cultures growing radishes. The experiments of HUTCHINSON and MILLER[7] with water cultures were doubtful. One of their pea cultures showed that the nitrogen content of the plant was increased by the use of glycocoll, although a slight decrease was noticed in another culture.

The experiments of BOROWIKOW[8] with *Helianthus* indicate that glycocoll retards growth. DACHNOWSKI and GORMLEY,[9]

[3] SCHREINER, O., and SKINNER, J. J., The effect of guanidine on plants. Bull. Torr. Bot. Club 39:535. 1912.

[4] SCHREINER, O., REED, H. S., and SKINNER, J. J., Certain organic constituents of soils in relation to soil fertility. Bull. 47. Bur. Soils, U.S. Dept. Agric. 1907.

[5] HANSTEEN, B., Om Aeggehvidesynthese i den gronne phanerogame Plante. Vidensk. Skrift. no. 3. 1898; Über Eiweissynthese in grünen Phanerogamen. Jahrb. Wiss. Bot. 33:417. 1899.

[6] MOLLIARD, M., Recherches sur le utilization par les plantes supérieures de diverses substances organiques azotées. Bull. Soc. Bot. France 10:541. 1910.

[7] HUTCHINSON, H. B., and MILLER, N. H. J., The direct assimilation of inorganic and organic forms of nitrogen by higher plants. Centralbl. Bakt. 30:513. 1911.

[8] BOROWIKOW, G. A., Über die Ursachen des Wachstums der Pflanzen. Biochem. Zeitschr. 50:119. 1913.

[9] DACHNOWSKI, A., and GORMLEY, R., The physiological water requirements and growth of plants in glycocoll solutions. Amer. Jour. Bot. 1:174. 1914.

working with a number of plants in water cultures, have shown that glycocoll is generally beneficial. It is pointed out that the process of absorption of glycocoll is not connected with the transpirational water loss, but with the differential permeability of the absorbing root cells, with the efficiency of the nutrient metabolism characteristic of the plant, and the amount of water retained within the plants.

Glycocoll ($CH_2 \cdot NH_2 \cdot COOH$) is amidoacetic acid and is one of the simpler degradation products found where decomposition is occurring. This primary degradation product of protein appears in the decomposition of plant remains and exists in considerable quantities in the tissue and seed of many plants. It is obtained synthetically by chemical processes in a number of ways.

Methyl glycocoll ($CH_2 \cdot NH \cdot CH_3 \cdot COOH$) differs in its chemical structure from glycocoll, not only in that it contains the methyl group CH_3, but also in that the amido group NH_2 is thereby changed to an imido group NH. Methyl glycocoll, as well as glycocoll, is a nitrogen-containing body; it is made synthetically and is not a simple protein body.

Effect of glycocoll

In this investigation the effect of glycocoll on growth in culture solutions was studied by growing wheat seedlings in solutions of calcium acid phosphate, sodium nitrate, and potassium sulphate. A large number of cultures was used; some consisted of the salts used singly, some of combinations of two salts, and others of all three salts used in different proportions. The total number of combinations used can be obtained from table IV, and from the discussion of the other tables. The composition of the solutions is given in the respective tables. Each solution was contained in a wide-mouth bottle holding 250 cc., and the solution was changed every three days, the old being replaced by fresh solution. The solutions were prepared by dissolving definite amounts of salts in carbon-treated distilled water. Two bottles of each solution were prepared, one to serve as a control, while to the other was added 50 ppm. of glycocoll. Each culture jar grew 10 wheat seedlings, supported in notched corks. The seedlings were placed in

the solutions when about 2 cm. tall, and grew for 12 days. The removal of salts was studied by analyzing the solutions for phosphate, nitrate, and potash, and the growth was observed and compared with its control, and the weight of green plants taken at the end of the experiment. The plants grew in these solutions from February 7 to February 19, 1914.

As the plants grew in the solutions, it was apparent that the glycocoll affected growth differently in the solutions containing different nutrient salts. The growth was increased much more by glycocoll in the solutions which contained no sodium nitrate than in those which contained large amounts of nitrate, as compared with the respective controls. This will be apparent from the weights of the green plants given in the accompanying tables.

TABLE I

EFFECT OF GLYCOCOLL IN CULTURE SOLUTIONS CONTAINING VARYING AMOUNTS OF PHOSPHATES AND POTASH AND NO NITRATE

Composition of culture solutions			Green weight	
P_2O_5	NH_3	K_2O	Without glycocoll	With 50 ppm. glycocoll
ppm.	ppm.	ppm.	gm.	gm.
80	0	0	1.01	1.05
72	0	8	1.26	1.55
64	0	16	1.53	1.85
56	0	24	1.50	1.95
48	0	32	1.55	2.05
40	0	40	1.47	2.00
32	0	48	1.39	2.05
24	0	56	1.50	1.95
16	0	64	1.65	1.70
8	0	72	1.40	1.90
0	0	80	1.37	1.65

Table I gives the green weight of the plants in solutions without and with glycocoll. The first three columns give the composition of each solution in parts per million P_2O_5 as calcium acid phosphate, NH_3 as sodium nitrate, and K_2O as potassium sulphate. The fourth column gives the green weight of the plants grown in solutions without glycocoll, and the last column the weight of the plants grown in solutions with 50 ppm. glycocoll.

An examination of the table shows that glycocoll, in those solutions which contained no nitrate, but varying amounts of

phosphate and potash, produced an increased growth in every culture. The total weight of the 11 normal cultures was 15.63 grams, and the weight of the glycocoll cultures was 19.70 grams, an increase of 26 per cent.

In table II are given the cultures which were composed of 8 ppm. of NH_3 as nitrate, and varying amounts of phosphate and potash.

TABLE II

EFFECT OF GLYCOCOLL IN NUTRIENT SOLUTIONS CONTAINING VARYING AMOUNTS OF PHOSPHATE AND POTASH AND 8 PPM. OF NH_3 AS NITRATE

Composition of culture solutions			Green weight	
P_2O_5	NH_3	K_2O	Without glycocoll	With 50 ppm. glycocoll
ppm.	ppm.	ppm.	gm.	gm.
72	8	0	1.48	1.65
64	8	8	1.73	2.05
56	8	16	2.20	2.09
48	8	24	2.40	2.43
40	8	32	2.37	2.70
32	8	40	2.34	2.35
24	8	48	1.30	1.45
16	8	56	2.20	2.25
8	8	64	2.35	2.40
0	8	72	1.90	2.05

The growth in this series of solutions was slightly better where glycocoll was added. An examination of the green weight columns of the table will show that nearly all the glycocoll cultures were slightly heavier. The total weight of the 10 cultures without glycocoll was 20.27 grams, against 21.42 grams for the cultures with glycocoll. This is an increase of 6 per cent.

The effect of glycocoll on growth in nutrient solutions containing still larger amounts of sodium nitrate is given in table III. These solutions, like the ones in the previous tables, are composed of varying amounts of phosphate and potash, but each contains 16 ppm. of NH_3 as nitrate.

In some of these solutions the glycocoll has slightly increased the growth, and in others the growth is slightly below the normal culture. The total weight of the 9 cultures without glycocoll was

20.75 grams, against 21.60 grams for the glycocoll, an average increase of only 4 per cent.

TABLE III

EFFECT OF GLYCOCOLL IN CULTURE SOLUTIONS CONTAINING VARYING AMOUNTS OF PHOSPHATE AND POTASH AND 16 PPM. NH_3 AS NITRATE

Composition of culture solutions			Green weight	
P_2O_5	NH_3	K_2O	Without glycocoll	With 50 ppm. glycocoll
ppm.	ppm.	ppm.	gm.	gm.
64	16	0	1.33	1.45
56	16	8	1.90	2.05
48	16	16	2.40	2.25
40	16	24	2.50	2.60
32	16	32	2.62	2.50
24	16	40	2.50	2.65
16	16	48	2.90	2.85
8	16	56	2.30	3.00
0	16	64	2.30	2.25

The beneficial effect of glycocoll in the solutions given in tables I, II, and III was most marked in those solutions containing no nitrate (table I). The effect was very slight in solutions containing 8 and 16 ppm. of NH_3 as nitrate, which indicates that the function of glycocoll in the nutrient solution is the same as that of nitrate; that is, that it seems to be absorbed by the plants and can take the place of nitrate in its effect on growth.

A large number of other solutions containing larger amounts of nitrate, up to 80 ppm., were employed in this experiment. No beneficial effect from glycocoll was observed in any of the cultures; on the other hand, some slight reductions in growth in some of these solutions was noted, but there was no marked harmful effect.

Absorption of nutrient salts as affected by glycocoll

The absorption of nutrients from the various solutions was determined, as mentioned before, by analyzing the cultures for nitrates immediately at the end of each 3-day period, and the phosphate and potassium on a composite of the solutions from the four changes. The colorimetric methods for determining small

amounts of salts, as described in Bulletin 70 of the Bureau of Soils, were employed in this investigation.

Considering first the absorption of phosphate, the entire set of cultures containing no glycocoll absorbed 177.5 mg. of P_2O_5, while the similar set of cultures with glycocoll absorbed 233 mg. The relative absorption of potash was somewhat similar. In the solutions without glycocoll, 613.7 mg. of K_2O was removed, while from the cultures with glycocoll 623.5 mg. was absorbed. With both phosphate and potash, the glycocoll cultures removed more than the normal cultures, which was to be expected, as the glycocoll cultures made a larger growth. However, the absorption of nitrate was less by the glycocoll than the normal cultures. The set of cultures containing no glycocoll removed 544.7 mg. NH_3, and the cultures with glycocoll only 320.5 mg. NH_3. The removal of less nitrate from solutions containing glycocoll is also contributing evidence that the plants use the glycocoll in building tissue, as it would use the nitrate in this particular function.

The effect of glycocoll seems to be the same as that of creatinine,[10] creatine, histidine, arginine,[11] asparagine,[12] xanthine, hypoxanthine, and nucleic acid,[13] all nitrogenous compounds and shown to be beneficial to growth, especially in the absence of any other form of nitrogen. These compounds replace the effect of nitrates on plants and are used as such by the plant.

In recent years it has been demonstrated that plants not only use nitrogen in the form of nitrates and ammonia, but that they can also use nitrogen in the form of complex organic compounds.[14] The

[10] Skinner, J. J., Beneficial effect of creatinine and creatine on growth. Bot. Gaz. 54:152. 1912.

[11] ———, Effect of histidine and arginine as soil constituents. Eighth Internat. Cong. Applied Chem. 15:253. 1912.

[12] ———, and Beattie, J. H., Effect of asparagine on absorption and growth. Bull. Torr. Bot. Club 39:429. 1912.

[13] Schreiner, O., and Skinner, J. J., Experimental study of the effect of some nitrogenous soil constituents on growth. Nucleic acid and its decomposition products. Plant World 16:45. 1913.

[14] Schreiner, O., Symposium on soils at the 1911 meeting of the A.A.A.S. Science 36:577. 1912.

Hutchinson, H. B., and Miller, N. H. J., The direct assimilation of inorganic and organic forms of nitrogen by higher plants. Centralbl. Bakt. 30:513. 1911.

Schreiner, O., and Skinner, J. J., Nitrogenous soil constituents and their bearing on soil fertility. Bull. 87. Bur. Soils, U.S. Dept. Agric. 1912.

action of these and a number of other nitrogenous compounds has been tested in this laboratory, and it has been found that these compounds are used as a source of nitrogen for the plant, without any transformation into ammonia, nitrites, or nitrates, and that the plant absorbs and uses them in preference to nitrate.

Effect of methyl glycocoll

The effect of methyl glycocoll on growth was studied in a similar way as was glycocoll; that is, wheat plants were grown in nutrient culture solutions composed of fertilizer salts, used singly and in combination of two and three salts. The methyl glycocoll was used in the cultures in amounts of 50 ppm., the same concentration as in the glycocoll experiments. The composition of the 66 nutrient solutions used are given in table IV, together with the green weight of the plants, grown in the solutions without and with methyl glycocoll. The plants grew from January 14 to January 26; within this time the solutions were changed and replaced by fresh solutions of the same composition four times in 3-day periods.

By an examination of table IV it will be seen that the methyl glycocoll, unlike the glycocoll, caused a decrease in growth. This is true in the entire set of cultures except four. The total green weight of the 66 cultures containing nutrient salts, but no methyl glycocoll, was 128.03 grams; while the total green weight of the similar set of cultures containing methyl glycocoll was 99.3 grams, a reduction in growth of 33 per cent.

The root growth of the plants in the methyl glycocoll solutions was shorter and did not have as healthy an appearance as the roots of the plants in the solutions which contained only the nutrient salts.

The tops, aside from being lighter in weight, were abnormal in appearance. The tops did not stand upright, but were twisted and grew in a lateral direction. This was true of each culture in the entire set, regardless of its content of nutrient salts. These physiologically disturbed plants had a pale green color, in contrast to the dark green of the normal cultures. The effect of this substance resembles somewhat that of cumarin,[15] which causes

[15] SCHREINER, O., and SKINNER, J. J., The toxic action of organic compounds as modified by fertilizer salts. BOT. GAZ. 54:31. 1912.

TABLE IV

EFFECT OF METHYL GLYCOCOLL ON GROWTH OF WHEAT SEEDLINGS IN CULTURE SOLUTIONS OF CALCIUM ACID PHOSPHATE, SODIUM NITRATE, AND POTASSIUM SULPHATE

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With 50 ppm. methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
1	80	0	0	1.02	0.95
2	72	0	8	1.27	1.30
3	72	8	0	1.32	1.20
4	64	0	16	1.32	1.25
5	64	8	8	1.47	1.45
6	64	16	0	1.20	1.20
7	56	0	24	1.35	1.30
8	56	8	16	1.80	1.55
9	56	16	8	1.85	1.45
10	56	24	0	1.35	1.20
11	48	0	32	1.15	1.30
12	48	8	24	1.95	1.40
13	48	16	16	2.25	1.70
14	48	24	8	1.75	1.45
15	48	32	0	1.60	1.10
16	40	0	40	1.47	1.25
17	40	8	32	2.07	1.55
18	40	16	24	2.19	1.75
19	40	24	16	2.14	1.40
20	40	32	8	1.95	1.50
21	40	40	0	1.60	1.15
22	32	0	48	1.52	1.35
23	32	8	40	2.12	1.50
24	32	16	32	2.42	1.75
25	32	24	24	2.44	1.70
26	32	32	16	2.02	1.72
27	32	40	8	1.90	1.60
28	32	48	0	1.40	1.30
29	24	0	56	1.42	1.32
30	24	8	48	2.09	1.82
31	24	16	40	2.44	1.84
32	24	24	32	2.22	1.80
33	24	32	24	2.42	1.80
34	24	40	16	2.32	1.70
35	24	48	8	1.89	1.40
36	24	56	0	1.49	1.42
37	16	0	64	1.47	1.32
38	16	8	56	2.30	1.42
39	16	16	48	2.55	1.70
40	16	24	40	2.54	1.59
41	16	32	32	2.54	1.70
42	16	40	24	2.24	1.60
43	16	48	16	2.37	1.55
44	16	56	8	1.97	1.57
45	16	64	0	1.70	1.40
46	8	0	72	1.57	1.37
47	8	8	64	2.07	1.59

TABLE IV—*Continued*

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With 50 ppm. methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
48	8	16	56	2.34	1.89
49	8	24	48	2.54	1.70
50	8	32	40	2.90	1.70
51	8	40	32	2.42	1.65
52	8	48	24	2.45	1.60
53	8	56	16	2.22	1.70
54	8	64	8	2.32	1.70
55	8	72	0	1.52	1.45
56	0	0	80	1.32	1.43
57	0	8	72	1.95	1.45
58	0	16	64	2.29	1.50
59	0	24	56	2.27	1.55
60	0	32	48	2.30	1.65
61	0	40	40	2.25	1.65
62	0	48	32	2.20	1.67
63	0	56	24	2.32	1.67
64	0	64	16	1.96	1.50
65	0	72	8	1.76	1.40
66	0	80	0	1.35	1.28

distorted stems and produces broad twisted leaves. Cumarin, however, does not cause a pale green leaf when grown in similar nutrient solution as does the methyl glycocoll. A set of normal and methyl glycocoll cultures is shown in figs. 1 and 2. The characteristic action is distinctly shown in the plants in fig. 2; the leaves are twisted and broad, which is in striking contrast to the straight upright plants of the normal cultures in fig. 1.

In analyzing the green weight figures given in table IV, it is interesting to note that the methyl glycocoll has reduced growth more in some of the cultures than in others, which will be brought out in the tables that follow. A close examination shows that the reduction was most in those solutions which normally produce the largest growth; that is, the difference between the green weight of specific cultures of some compositions without and with methyl glycocoll is greater where the growth is greater in the solutions containing only the nutrient salts.

The 66 cultures given in table IV permit of better discussion when they are arranged according to the triangular scheme upon which their composition depends. This scheme has been fully

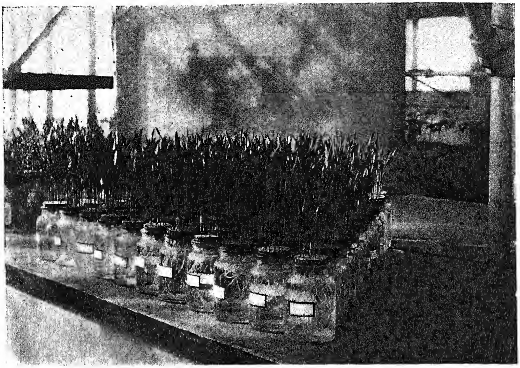

FIG. 1.—Wheat plants growing in nutrient solutions

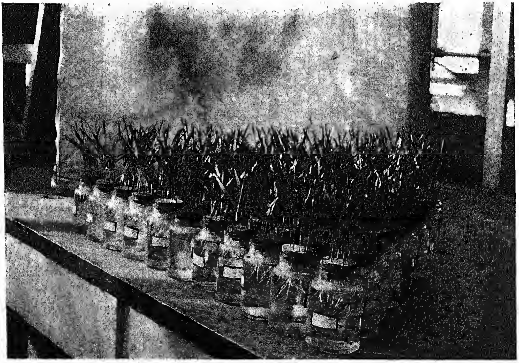

FIG. 2.—Wheat plants growing in nutrient solutions with 50 ppm. of methyl glycocoll.

presented in an earlier paper[16] and needs no further exposition here. As shown in this earlier paper, the maximum growth takes place in the middle region of the lower part of the diagram; that is, in those cultures which contain phosphate between the limits 8–24 ppm., and nitrate and potash between the limits 24–48 ppm. These are the cultures represented by group *a* in fig. 3, which is here

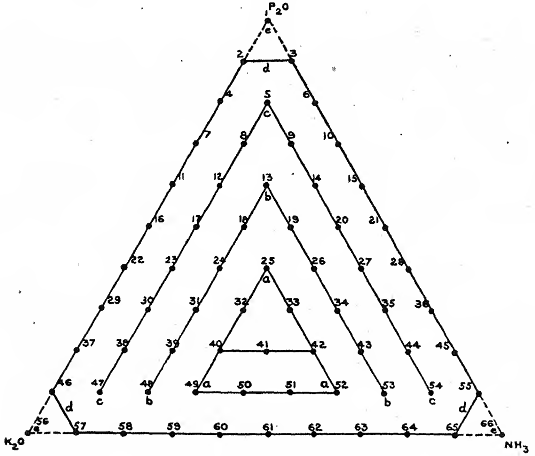

FIG. 3.—The arrangement of the culture solutions in groups: *a, b, c*, three fertilizer element groups; *d*, two fertilizer element group; *e*, one fertilizer element group.

again reproduced to make the discussion clear. It has been shown that this group *a* has the largest growth, and the greatest absorption of nutrients by the growing plants also takes place in this group. Both growth and absorption become less successively in groups *b, c*, and *d*. The latter group contains only two of the fertilizer elements in any one solution, and the growth in these is

[16] SCHREINER, O., and SKINNER, J. J., Ratio of phosphate, nitrate, and potassium on absorption and growth. BOT. GAZ. 50:1. 1910.

considerably poorer than in those containing three of the fertilizer elements.

If we consider now the results of table IV in the light of these facts and in the same groupings, some interesting facts regarding the effect of the methyl glycocoll are revealed.

In table V are given the cultures of group *a*, which as a group normally produces the largest growth and the greatest absorption. The number of the culture is given in the first column; the third, fourth, and fifth columns give the composition of the solution; the sixth the green weight of plants grown in solution without methyl glycocoll; and the last column the green weight of plants with methyl glycocoll. There is considerable difference in growth in these two sets of cultures, as is seen in the table.

TABLE V

GROWTH OF WHEAT PLANTS IN THE BEST CULTURE SOLUTIONS OF GROUP *a* WITHOUT AND WITH METHYL GLYCOCOLL

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
25	32	24	24	2.44	1.70
33	24	24	32	2.22	1.80
34	24	32	24	2.42	1.80
40	16	24	40	2.54	1.59
41	16	32	32	2.54	1.70
42	16	40	24	2.24	1.60
49	8	24	48	2.54	1.70
50	8	32	40	2.90	1.70
51	8	40	32	2.42	1.65
52	8	48	24	2.45	1.60

The total green weight of the 10 cultures without methyl glycocoll was 24.71 grams, against 16.84 grams for the methyl glycocoll cultures, a reduction of 32 per cent. It is also interesting to compare the absorption of phosphate, nitrate, and potassium in these two sets of cultures. The 10 normal cultures absorbed 28.9 mg. of P_2O_5, NH_3, and K_2O, and the 10 cultures with the methyl glycocoll absorbed 20.8 mg. That is, the methyl glycocoll cultures absorbed 28 per cent less nutrients than the normal cultures.

We will next consider the solutions of group *b*, which contained more unequal ratios and which have previously proved to have a poorer physiological influence on plants as manifested by the growth. The composition of these solutions together with the green weights of the two sets of cultures is given in table VI.

TABLE VI

GROWTH OF WHEAT PLANTS IN CULTURE SOLUTIONS OF GROUP *b* WITHOUT AND WITH METHYL GLYCOCOLL

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
13..........	48	16	16	2.25	1.70
18..........	40	16	24	2.19	1.75
19..........	40	24	16	2.14	1.40
24..........	32	16	32	2.42	1.75
26..........	32	32	16	2.02	1.72
31..........	24	16	40	2.44	1.84
34..........	24	40	16	2.32	1.70
39..........	16	16	48	2.55	1.70
43..........	16	48	16	2.37	1.55
48..........	8	16	56	2.34	1.89
53..........	8	56	16	2.22	1.70

The figures show that methyl glycocoll produced less growth than the normal solutions. The green weight of the 11 normal cultures was 26.10 grams, against 18.70 grams total green weight for the methyl glycocoll cultures, a reduction of 29 per cent due to the methyl glycocoll. The 11 normal cultures absorbed 27.43 mg. of P_2O_5, NH_3, and K_2O, while the methyl glycocoll cultures absorbed 19.45 mg., a reduction of 22 per cent in nutrients absorbed. It is interesting to note that methyl glycocoll reduced both the growth and absorption less in these cultures of group *b* than in those of group *a* given in table V.

In table VII is given the effect of methyl glycocoll in the solutions of group *c*, which have been shown to be not as suitable for plant development as those given in the two preceding tables.

The total green weight of the 15 cultures without methyl glycocoll was 29.50 grams, and the total green weight of the same solutions containing methyl glycocoll was 22.95 grams, a reduction

of 22 per cent in growth. The absorption of P_2O_5, NH_3, and K_2O from these solutions for the 15 normal cultures was 18.2 mg., against 15.5 mg. for the total number of cultures containing methyl glycocoll, a reduction of 15 per cent. Again it is seen that in these cultures where absorption and growth is small the methyl glycocoll is less harmful.

TABLE VII

GROWTH OF WHEAT PLANTS IN CULTURE SOLUTIONS OF GROUP *c* WITHOUT AND WITH METHYL GLYCOCOLL

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
5	64	8	8	1.47	1.45
8	56	8	16	1.80	1.55
9	56	16	8	1.85	1.45
12	48	8	24	1.95	1.40
14	48	24	8	1.75	1.45
17	40	8	32	2.07	1.55
20	40	32	8	1.95	1.50
23	32	8	40	2.12	1.50
27	32	40	8	1.90	1.60
30	24	8	48	2.09	1.82
35	24	48	8	1.89	1.40
38	16	8	56	2.30	1.42
44	16	56	8	1.97	1.57
47	8	8	64	2.07	1.59
54	8	64	8	2.32	1.70

In table VIII are given the green weights of plants grown in solution containing only two nutrient salts (group *d*), with and without methyl glycocoll. Solutions containing only two salts produce less growth than solutions containing three salts, in any proportion.

The total growth of the 27 cultures without methyl glycocoll was 44.72 grams green weight, against 37.32 grams for the cultures with methyl glycocoll, a reduction of only 16 per cent.

From tables V, VI, VII, and VIII it is apparent that growth was greatest in the cultures of group *a*, and that the greatest amount of absorption took place in these solutions. The harmful effect of methyl glycocoll was also most marked in this group of solutions. A comparison of tables VI, VII, and VIII shows that those solutions

in which less absorption took place and less growth was produced, the effect of the organic compound was also less.

TABLE VIII

GROWTH OF WHEAT PLANTS IN CULTURE SOLUTIONS OF GROUP *d* COMPOSED OF TWO FERTILIZER ELEMENTS, WITHOUT AND WITH METHYL GLYCOCOLL

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
2	72	0	8	1.27	1.30
4	64	0	16	1.32	1.25
7	56	0	24	1.35	1.30
11	48	0	32	1.15	1.30
16	40	0	40	1.47	1.25
22	32	0	48	1.52	1.35
29	24	0	56	1.42	1.32
37	16	0	64	1.47	1.32
46	8	0	72	1.57	1.37
57	0	8	72	1.95	1.45
58	0	16	64	2.29	1.50
59	0	24	56	2.27	1.55
60	0	32	48	2.30	1.65
61	0	40	40	2.25	1.65
62	0	48	32	2.20	1.67
63	0	56	24	2.32	1.67
64	0	64	16	1.96	1.50
65	0	72	8	1.76	1.40
55	8	72	0	1.52	1.45
45	16	64	0	1.70	1.40
36	24	56	0	1.49	1.42
28	32	48	0	1.40	1.30
21	40	40	0	1.60	1.15
15	48	32	0	1.60	1.10
10	56	24	0	1.35	1.20
6	64	16	0	1.20	1.20
3	72	8	0	1.32	1.20

In another experiment the wheat plants grew in solutions of glycocoll and methyl glycocoll, side by side at the same time, from February 22 to March 4. In this test only 11 different nutrient solutions were employed. One set of 11 cultures was used as a control; to each culture of the second set of 11 cultures 50 ppm. of glycocoll was added, and to the third set methyl glycocoll in the same amounts was added. The result of this test was very much the same as observed in the preceding experiments. The glycocoll produced increased growth in those solutions which contained no

nitrate and in those which had a small amount of nitrate, but only a very small increase in the solutions which contained larger amounts of mineral nitrate. The methyl glycocoll produced a stunted growth and a peculiar twisting and lateral growth of the top of the plant, as before noted. There was a 25 per cent decrease in growth from the control cultures.

Effect of calcium carbonate on the action of methyl glycocoll

An experiment was made to study the effect of methyl glycocoll under alkaline conditions. In this case 11 nutrient solutions were

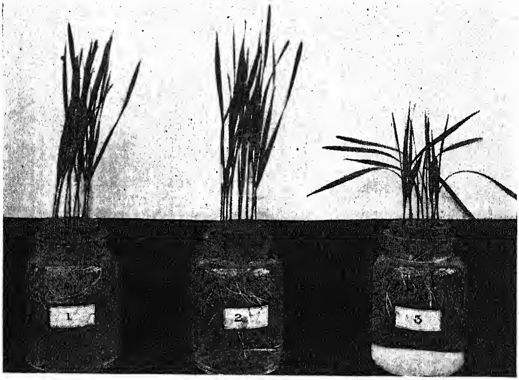

FIG. 4.—Effect of glycocoll and methyl glycocoll on wheat plants in a nutrient solution containing 8 ppm. P_2O_5, 22 ppm. NH_3, and 48 ppm. K_2O; no. 1, nutrient solution; no. 2, same with glycocoll; no. 3, same with methyl glycocoll and calcium carbonate.

used, as in the experiment just recorded; one set of 11 was used as a control; another set contained glycocoll; and a third set methyl glycocoll with calcium carbonate. The physiological effect of the methyl glycocoll, in solutions with calcium carbonate, was the same as in the solutions already discussed. The effect of both the

glycocoll and methyl glycocoll is shown in fig. 4. The effect of the compounds was not materially different in the solutions of different composition. No. 1 is the control culture, no. 2 in addition to the nutrient salts contains 50 ppm. of glycocoll, and no. 3 contains 50 ppm. of methyl glycocoll with calcium carbonate. It will be observed that the plants in the glycocoll cultures are slightly larger than culture no. 1, the control. The characteristic effect of methyl glycocoll is shown in the third culture. The photograph shows the growth to be smaller and the stems twisted and leaves growing almost horizontally. The growth in the 11 cultures containing methyl glycocoll was 27 per cent less than in the control set, which shows that lime has not changed its physiological action.

Soil Fertility Investigations
Department of Agriculture
Washington, D.C.

THE EFFECT OF SOME TRIVALENT AND TETRAVALENT KATIONS ON PERMEABILITY

W. J. V. OSTERHOUT

(WITH SEVEN FIGURES)

It has been shown[1] that there is a remarkable difference between monovalent and bivalent kations in their effects on permeability. None of the monovalent kations investigated (except H) are able to decrease permeability, while all of the bivalent kations investigated are able to do so to a marked degree. In view of this it becomes important to make similar investigations on the effects of trivalent and tetravalent kations.

It is desirable in these investigations to use salts which give neutral solutions, since, as has been previously shown,[2] both acid and alkali affect permeability. For this reason salts of lanthanum are especially useful; nitrates of yttrium and cerium were also employed, as they likewise give neutral solutions when used as here described. Some experiments were made with ferric sulphate and with aluminum salts, but these substances have the disadvantage of giving acid solutions.

The salts used were in all cases the purest obtainable and the distilled water was prepared with especial care.

A solution of $La_2(NO_3)_6 \cdot 12\ H_2O$ of the conductivity of sea water was made by dissolving 31.5 gm. in 297 cc. of distilled water. The concentration was about 0.126 M. A lot of tissue which had a resistance in sea water of 1350 ohms was transferred to the lanthanum solution. The resistance rose rapidly to a maximum of 2350 ohms after which it gradually fell. In a second experiment the resistance at the start was 880 ohms and the maximum resistance 1490 ohms. The results are shown in table I and fig. 1.

[1] OSTERHOUT, W. J. V., On the decrease of permeability due to certain bivalent kations. BOT. GAZ. 59:315–330. *figs. 11.* 1915.

[2] ———, Extreme alterations of permeability without injury. BOT. GAZ. 59:242–253. *figs. 4.* 1915.

At the beginning of the first experiment the resistance was 1350 ohms; from this we must substract the resistance of the apparatus (240 ohms) to get the resistance of the tissue itself or the net resistance. This was $1350-240=1110$ ohms, and the net conductance $1\div 1110=0.000901$ mho. The net resistance at the maximum was $2350-240=2110$ ohms, and the net conductance was $1\div 2110=0.000474$ mho. We may regard the permeability as equal to the conductivity, or for convenience we may, in such a case as this, regard it as equal to the conductance. The loss in permeability therefore was $0.000901-0.000474=0.000427$ mho or 47.4 per cent.

TABLE I

ELECTRICAL RESISTANCE OF *Laminaria saccharina;* TWO EXPERIMENTS

Time in hours	In $La_2(NO_3)_6$ 0.126 M	In $CaCl_2$ 0.278 M	In sea water
0	1350	1300	1400
0.17	2080	1640	....
0.33	2160	1730	....
1	2350	1730	....
1.83	2315	1640	....
3.33	2030	1490	1400
10	1340	600	1370
18	880	400	1350
0	880	....	890
0.5	1490	....	....
1	1360	....	....
2	1320	....	....
5.5	740	....	....
9.5	525	....	890

All readings were taken at 18° C.

The net resistance at the beginning of the second experiment was $880-250=630$ ohms, and the net conductance was $1\div 630=0.00159$ mho. The net resistance at the maximum was $1490-250=1240$ ohms, and the net conductance was $1\div 1240=0.00081$ mho. The loss in permeability therefore was $0.00159-0.00081=0.00078$ mho or 49.1 per cent. Similar results were obtained with La_2Cl_6. It will be seen that the rise in resistance is much greater in $La_2(NO)_6$ and La_2Cl_6 than in $CaCl_2$.

It has been pointed out[1] that the severest test of the ability of a salt to decrease permeability is to add the salt in solid form to the

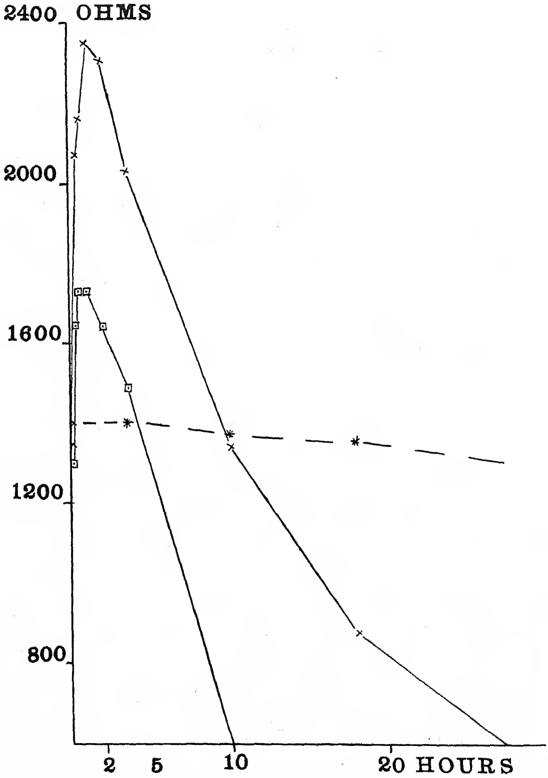

FIG. 1.—Curves of electrical resistance of *Laminaria saccharina* in $La_2(NO_3)_6$ 0.126 M (line with crosses), in $CaCl_2$ 0.278 M (line with squares), and in sea water (dotted line).

sea water. The results of such an experiment are shown in table II and fig. 2. To 275 cc. of sea water 5 gm. $La_2(NO_3)_6 \cdot 12\ H_2O$ were

TABLE II

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 275 cc.+ $La_2(NO_3)_6 \cdot 12\ H_2O$ 5 gm. (=0.021 M)	In sea water
0	1090	1080
0.08	1210	1080
0.75	1210	....
1.25	1210	....
2	1190	....
17	980	1030
22.5	935	1000

All readings were taken at 18° C.

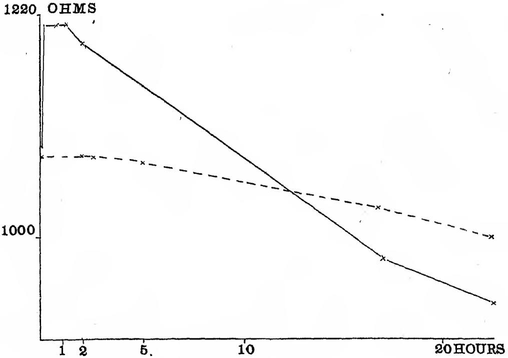

FIG. 2.—Curve of electrical resistance of *Laminaria saccharina* in sea water 275 cc.+$La_2(NO_3)_6 \cdot 12\ H_2O$ 5 gm. (=0.021 M) (unbroken line), and of a control in sea water (dotted line).

added, making the concentration 0.021 M. In this the resistance of a lot of tissue rose rapidly from 1090 to 1210 ohms, where it remained stationary for a time and then began to fall. In the course

of 17 hours it fell to 980 ohms, while that of the control fell 50 ohms in the same time. Dead tissue gave no rise in resistance.

TABLE III

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 300 cc.+ $Ce_2(NO_3)_6 \cdot 12\ H_2O$ 0.8 gm.=(0.003 M)	In sea water
0	840	810
0.16	860	....
0.5	970	....
1.5	990	....
4	860	800
19	420	770

All readings were taken at 18° C.

The addition of the salt in solid form increases the conductivity of the solution. In order to produce a rise in resistance when added in this way, the action of the salt must be great enough to overcome the fall in the resistance of the solution which is contained in the apparatus and in the intercellular substance[3] of the tissue.

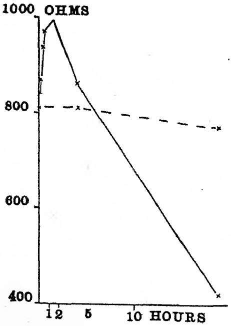

FIG. 3.—Curve of electrical resistance of *Laminaria saccharina* in sea water+$Ce_2(NO_3)_6 \cdot 12\ H_2O$ 0.8 gm. (=0.003 M) (unbroken line), and of a control in sea water (dotted line).

As has been pointed out,[1] such experiments furnish conclusive proof that the current passes through the protoplasm as well as through the intercellular substance.

In a previous paper the results of exposing the tissue alternately to sea water and to sea water+$La_2(NO_3)_6$ were described in detail. The experiment shows that repeated exposure to sea water+$La_2(NO_3)_6$ produces no injury.[4]

[3] The frond may be regarded as a mass of intercellular substance in which numerous masses of protoplasm (the cells) are imbedded.

[4] Science 36:350. 1912.

A lot of tissue which had in sea water a resistance of 840 ohms was placed in sea water to which $Ce_2(NO_3)_6 \cdot 12\ H_2O$ had been added (0.8 gm. to 300 cc. of sea water, making the concentration 0.003 M). In the course of 30 minutes the resistance rose to 970 ohms; during the next hour it continued to rise, reaching a maximum of 990 ohms, after which it slowly fell. The results are given in table III and fig. 3.

TABLE IV

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 300 cc.+ $Y(NO_3)_3 \cdot 6\ H_2O$ 0.8 gm. (=0.007 M)	In sea water
0	740	770
0.5	900	....
1.25	940	....
2	970	....
5	900	....
20.5	630	735

All readings were taken at 18° C.

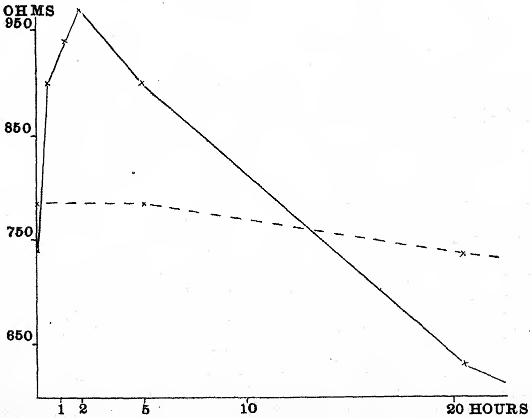

FIG. 4.—Curve of electrical resistance of *Laminaria saccharina* in sea water 300 cc. $+Y(NO_3)_3 \cdot 12\ H_2O$ 0.8 gm.=(0.007 M) (unbroken line), and of a control in sea water (dotted line).

A similar experiment was performed by adding 0.8 gm. $Y(NO_3)_3 \cdot 6H_2O$ to 300 cc. of sea water (=0.007 M) and placing a lot

TABLE V

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc.+ $Fe_2(SO_4)_3$ 1 gm. (=0.0025 M)	In sea water
0	750	730
0.5	810	.....
1.5	600	.....
17	500	670

All readings were taken at 18° C.

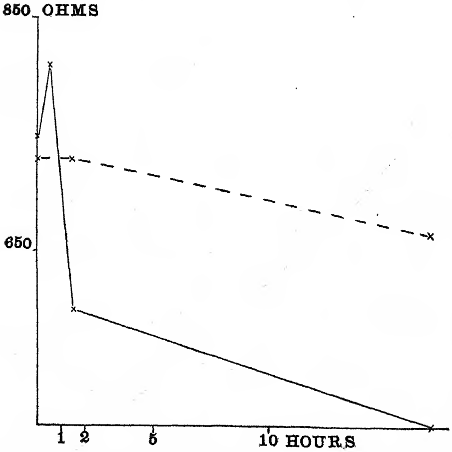

FIG. 5.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+$Fe_2(SO_4)_3$ (=0.0025 M) (unbroken line), and of a control in sea water (dotted line).

of tissue in this mixture.[5] The tissue had in sea water a resistance of 740 ohms; after being transferred to sea water+$Y(NO_3)_3$ the

[5] It should be noted that if the dissociation were equal, a molecule of $Y_3(NO_3)_3$ would yield only half as many kations as a molecule of $La_2(NO_3)_6$ or of $Ce_2(NO_3)_6$.

resistance rose in 30 minutes to 900 ohms, and in the course of 2 hours reached 970 ohms; it then began to fall, and at the end of

TABLE VI

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc.+ $Al_2(SO_4)_3$ 18 H_2O 6.7 gm.	In sea water
0	800	840
0.5	1100	....
0.75	1000	....
1.25	950	....
6.25	370	800

20.5 hours it was 630 ohms. The results are shown in table IV and fig. 4 (p. 469).

Another lot of tissue which had in sea water a resistance of 750 ohms was transferred to sea water 1000 cc.+ $Fe_2(SO_4)_3$ 1 gm. (=0.0025 M). The resistance rose in the course of 30 minutes to 810 ohms; at the end of 1.5 hours it had fallen to 600 ohms, and it continued to fall rapidly after this. The solution was acid to litmus, but the degree of acidity was not sufficient to account for the whole of the effect. The results are shown in table V and fig. 5.

Experiments were made with several salts of aluminum, including aluminum chloride, aluminum sulphate, ordinary alum, and chrome alum, which were added in solid form to sea water. All of them gave similar results. The solutions were acid, but the acidity was not great enough to account for the whole of the effect. The action of these salts is illustrated by table VI and fig. 6, which show the results obtained by adding 6.7 gm. $Al_2(SO_4)_3 \cdot 18\ H_2O$ to

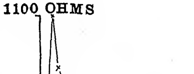

FIG. 6.—Curves of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+$Al_2(SO_4)_3 \cdot 18\ H_2O$ 6.7 gm. (=0.01 M) (unbroken line), and of a control in sea water (dotted line).

1000 cc. sea water (=0.01 M). It is evident that aluminum salts are very toxic.

As the result of plasmolytic investigations, FLURI[6] came to the conclusion that salts of aluminum (also of lanthanum and of

TABLE VII

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 300 cc.+ Th $(NO_3)_4$ 0.4 H_2O 1 gm. (=0.006 M)	In sea water
0	690	720
0.5	750	....
1	760	....
2.5	680	....
5	630	720
7.5	600	....
29	540	660
40	510	640

All readings were taken at 18° C.

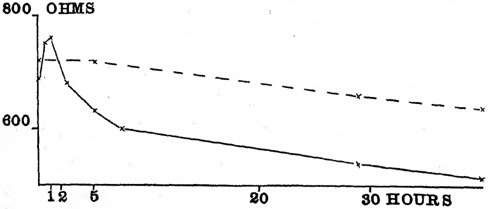

FIG. 7.—Curve of electrical resistance of *Laminaria saccharina* in sea water 300 cc. +Th $(NO_3)_4 \cdot 4\ H_2O$ 1 gm. (=0.006 M) (unbroken line), and of a control in sea water (dotted line).

yttrium) increase protoplasmic permeability. SZÜCS[7] made experiments on the taking up of dyes by the cell and concluded that salts of aluminum decreased the permeability. The experiments described show that both effects are produced. Which effect predominates depends both on the concentration of the salt (as is shown by experiments not mentioned here) and on the length of exposure to its action.

[6] Flora 99:81. 1908.

[7] Sitzungsber. Wiener Akad. 119: 1910.

In order to observe the effect of a tetravalent kation, 1 gm. $Th(NO_3)_4 \cdot 4\ H_2O$ was added to 300 cc. sea water, making the concentration 0.006 M. Tissue which had in sea water a resistance of 690 ohms was placed in this solution; the resistance rose in the course of half an hour to 750 ohms; at the end of one hour it was 760 ohms; after this it fell slowly, and at the end of 40 hours was 510 ohms. The results are shown in table VII and fig. 7.

Summary

All of the trivalent kations investigated (La, Ce, Y, Fe, Al) and the tetravalent kation Th are able to decrease permeability to a marked degree.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

PHYSIOLOGICAL ISOLATION OF TYPES IN THE GENUS XANTHIUM

CHARLES A. SHULL

(WITH SEVEN FIGURES)

While collecting *Xanthium* seeds for physiological studies, my attention has often been called to the heterogeneity of the *Xanthium* population in the field. Burs collected from several widely separated localities belong to the uncertain group listed under the name of *X. canadense* Miller in ROBINSON and FERNALD'S seventh edition of GRAY'S *Manual.*

In the annual report of the botanist of the Department of Agriculture for 1886, VASEY mentions *X. canadense* as the species troublesome in the west, while the principal eastern species is called *X. strumarium.* But it is now known that *X. strumarium* has never been introduced into America. As several types were often found growing intermingled in Kansas and Kentucky, the possibility of hybridization suggested itself.

Desiring seeds of uniform physiological character for certain investigations in which great accuracy was necessary, I collected burs from the three main types occurring in the fields about Lawrence, Kansas. In each case the seeds were chosen from a single plant of the type. It was thought that the various forms were possibly the result of promiscuous crossing of varieties or elementary species, and that a year or two of guarded pollination would be necessary to purify the strains so that physiological properties as well as morphological characters might be uniform. The use of pure bred material for physiological investigations has not yet been considered essential, but it may be very desirable, or even necessary, for certain kinds of work. Burs from the three types chosen are shown in figs. 1, 2, and 3. The original plants stood side by side on the northern edge of the Wakarusa floodplain about 0.5 kilometer south of Mount Oread. After being photographed, the burs were opened, and the seeds of the three types were found

to differ (fig. 4). The type with globose burs has much shorter seeds than the other two types, which are evidently more closely related. The seeds from type I have a dark brown testa, while those of type II have a dull greenish or grayish brown testa, and type III a yellowish brown testa.

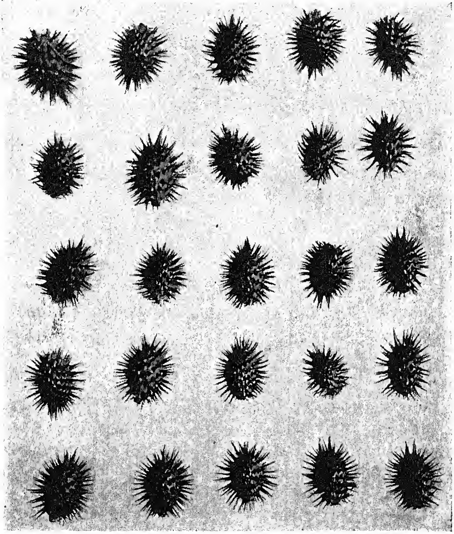

FIG. 1.—Type I, *Xanthium globosum* Shull, sp. nov.; natural size

The variation in size and weight of the seeds of the several types is under investigation. The average weight of 50 upper seeds of type I in the crop of 1913, an excessively dry year, is about 20 mg., of the lowers 27 mg. The corresponding average weights for type II are 28 mg. and 48 mg. respectively. The curves of variability in weight and length will probably show some overlapping in the

two types when a larger series has been measured, but it is certain to be slight.

Seedlings raised in the laboratory were transferred to the breeding grounds about June 1, 1913. Typical seedlings of types I and II are shown in fig. 5. The cotyledons of type I are somewhat

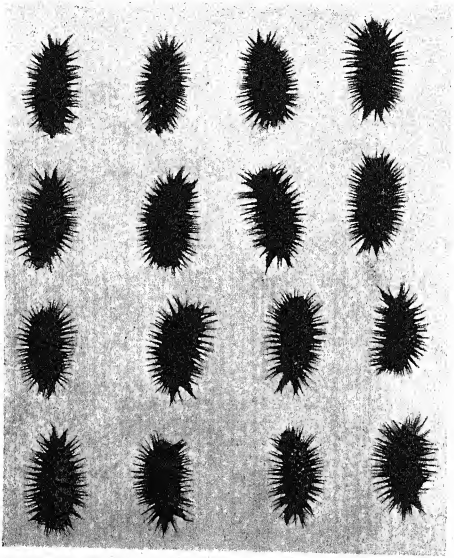

FIG. 2.—Type II, *Xanthium pennsylvanicum* Wallr.; natural size

shorter and broader than those of type II, which are long and strap-shaped; but the difference is not as great as would have been expected from the difference in the seeds.

The three types were planted within a day of each other; type I first, type II the following day, and type III last. As the plants

began to develop their characteristic mature leaves, a very surprising uniformity of the plants belonging to each type was observed. This result was wholly unexpected, as it was believed that hybridization could hardly have been avoided in nature. Type I has a dark green mesophyll with veins almost white, and a much

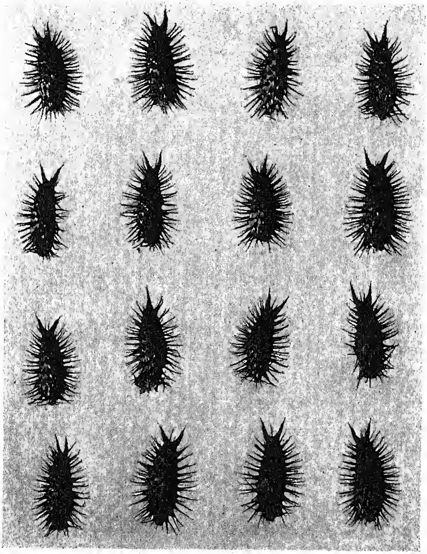

Fig. 3.—Type III, *Xanthium canadense* Mill.; natural size

crinkled surface; while type II has yellow green foliage, the leaf surfaces relatively plane. The foliage of type III resembles that of type I very closely, so that it is difficult to distinguish young plants (figs. 6 and 7 show the foliage differences of types I and II).

The most interesting difference between the two types is shown also in these figures. The photographs were taken at the same time, about September 1, 1913. Type II had shed all of its pollen and its burs were full grown. One shriveled cluster of staminate flowers can be seen above; while type I is just beginning to open its first staminate flowers, and its carpellate flowers are almost too small to be seen. Type III was intermediate, having about half

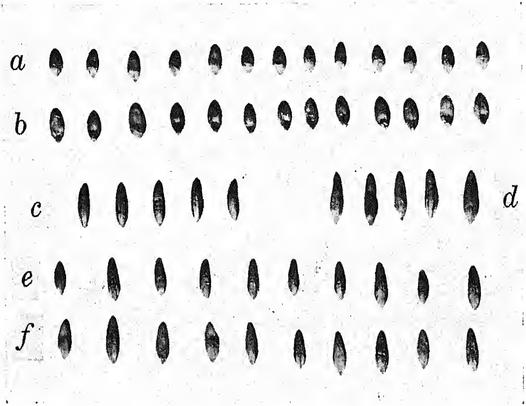

FIG. 4.—*a* and *b*, uppers and lowers of *X. globosum* Shull; *c* and *d*, uppers and lowers of *X. pennsylvanicum* Wallr.; *e* and *f*, uppers and lowers of *X. canadense* Mill.; all natural size.

grown burs at the time type I sheds its pollen; its flowers had no doubt been generally pollinated from plants of the same type. In this interesting condition we find the explanation of the remarkable uniformity exhibited by the offspring of plants taken at random from a heterogeneous population in the field. There is a physiological isolation that effectually prevents hybridization in the great majority of cases. The pollen of one variety has been shed long before the stigmas of the other are ready for the pollination processes.

During the summer of 1914, volunteer seedlings of types I and II were allowed to grow in the field under natural conditions, in competition with plants of the same types and other native weeds. The same differences in the burs and in the blooming times occurs under these circumstances, but the differences in the foliage loses some of its sharpness due to type I showing less crinkling of the mesophyll when crowded. There are wide differences

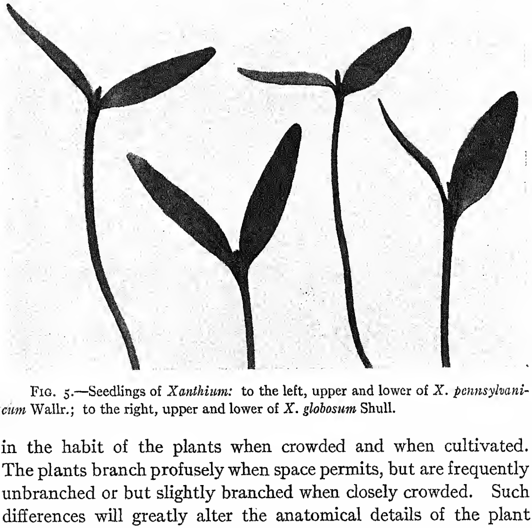

Fig. 5.—Seedlings of *Xanthium:* to the left, upper and lower of *X. pennsylvanicum* Wallr.; to the right, upper and lower of *X. globosum* Shull.

in the habit of the plants when crowded and when cultivated. The plants branch profusely when space permits, but are frequently unbranched or but slightly branched when closely crowded. Such differences will greatly alter the anatomical details of the plant structure, and tend to render such distinctions uncertain.[1]

One more difference between the three types remains to be noted. Type II develops considerable anthocyanin in the prickles

[1] The comparative anatomy of these three types of *Xanthium*, with *X. canadense* going under the name of *X. americanum*, has been described by Nora E. Dalbey (Kan. Univ. Sci. Bull. 9:57–65. 1914).

and hairs that beset the burs, so that the rows of plants appear reddish brown long before the burs begin to dry. Type II develops much less anthocyanin, while pigment is entirely wanting in type I. When the burs have dried this difference is manifest in different shades of brown. The burs are lightest in color in type I, darkest in type II.

FIG. 6.—*X. pennsylvanicum* Wallr., at the same age as *X. globosum* Shull in fig. 7

Since the three types tend to remain distinct in the field, and since all of them show distinctive features while presenting no definite evidence of Mendelian segregation, they should be considered distinct species.

The genus *Xanthium* is in need of careful study, and possibly revision, on the basis of breeding tests. The principal difficulty in the genus from the taxonomic point of view is to interpret just what the old authors meant by *X. canadense* Mill. and *X. ameri-*

canum Walt. which was for a time called *X. strumarium* in the east, and which was redescribed by BRITTON in 1901 as *X. glabratum*. In the second edition of BRITTON and BROWN this species is now listed as *X. americanum* Walt. It seems clear from the descriptions that type III, with rather slender, oblong, essentially glabrous burs, should bear the name *X. canadense* Miller (Gard.

FIG. 7.—*X. globosum* Shull, at the same age as *X. pennsylvanicum* Wallr. in fig. 6

Dict. ed. 8. no. 2. 1768); with this species, *X. americanum* Walt. (1. Car. 231. 1788) and *X. glabratum* Britt. (BRITT. Manual 912. 1901) are synonymous.

Type II, with oblong pubescent fruit, agrees fairly well with the description of *X. pennsylvanicum* Wallr., and must on the basis

of breeding test be considered as a species distinct from *X. canadense* Miller.

The burs of type I do not resemble those of any of the 8 species now recognized in the territory east of the 100th meridian. This variety was first seen on the campus of the State University of Kentucky several years ago, where it formed a very small percentage of the cocklebur population. But here it was the most prominent variety in the field at the time the original selection was made in 1912. In addition, it is the most prolific type so far found. One specimen of average size bore 1864 burs, as compared to 75 on *X. speciosum*. It has been found to breed true, and since it is distinguished at once by its short smooth globose bur, I am naming it tentatively **Xanthium globosum.**

No study has yet been made of the geographical distribution and probable migrations of the several forms. The possibility suggests itself that *X. globosum* is a southern or southwestern form, developed in a region favorable to a long developmental period, and that *X. pennsylvanicum* is of more northern origin, with a correspondingly short period of growth. The two types may have met and mingled in the plains region without the possibility of a general hybridization because of the physiological isolation. An illustration of the behavior of a northern species may be cited in this connection. Seeds of *X. speciosum* Kearney obtained from South Dakota were planted about June 1, 1914, and had half grown burs by July 20, almost a full month before *X. pennsylvanicum* had developed its flowers, and six weeks before *X. globosum* was in bloom.

X. canadense Miller, as here interpreted, is in a number of ways intermediate between *X. globosum* and *X. pennsylvanicum*. It has an intermediate blooming period, intermediate sized burs and seeds, although the seeds stand much nearer to *X. pennsylvanicum* than to *X. globosum*, and intermediate production of anthocyanin. It has the oblong shape of *X. pennsylvanicum*, the glabrous character of the bur and the somewhat crinkly leaf of *X. globosum*. It is not yet known whether *X. canadense* can be produced by crossing *X. globosum* and *X. pennsylvanicum*, and selecting a pure form having the recombinations which it shows. While in general the

Xanthium population seems to be isolated according to species, there is always the possibility that the latest plants of one species may have the opportunity to cross with the earliest plants of a later blooming species. Such a cross between *X. globosum* and *X. pennsylvanicum* might possibly have given rise to *X. canadense*.

At a later time, when the range of variability of *X. globosum* has been carefully determined, the technical description of the species will be given. In the meantime the photographs will enable any one to identify the new species. There are probably a number of new species of *Xanthium* still undescribed in America, as would be indicated by the fact that half of the 8 species now recorded for the eastern half of the United States have been known less than sixteen years.

I am indebted to Mr. L. M. Peace of the botany department of the University of Kansas for the excellent photographs of the types, and especially to Dr. J. M. Greenman, curator of the herbarium of the Missouri Botanical Garden, St. Louis, for examining the photographs and comparing the materials with specimens in the herbarium, and for information regarding the synonymy of *X. americanum* and *X. glabratum* with *X. canadense* Miller.

University of Kansas
Lawrence, Kansas

THE ORIGIN AND DISTRIBUTION OF THE FAMILY MYRTACEAE

EDWARD W. BERRY

In the study of the lower eocene flora of the Mississippi embayment, a rather exhaustive compilation was undertaken to show the geological distribution of the genera present in that flora and the geographical distribution of the existing species in the families that were represented. This laborious work fully repaid the time involved, since many facts of general interest came to light and many highly suggestive, even if unproved, ideas emerged from the statistical tables.

A preliminary and somewhat tentative sketch of these matters was published,[1] and it is planned to elaborate the subject further in the final publication on the lower eocene flora under the auspices of the U.S. Geological Survey.

Among the various families that are represented in numerous fossil floras, none has excited greater interest among students of recent floras and geographical distribution than the family Myrtaceae, which in the genus *Eucalyptus* and its more immediate allies is so prominent an element in the present flora of Australia. What may be legitimately expected when fossil and recent floras shall have been studied in a sufficiently broad way, even with the present insufficient data of both recent and fossil geographical distribution, may be illustrated by the following brief sketch of what is known of the Myrtaceae.

The family Myrtaceae contains over 3100 existing species, separated by taxonomists into two subfamilies. The first of these, the Myrtoideae, with 32 genera and about 2400 existing species, comprises mostly tropical forms, of which over 75 per cent are confined to the Western Hemisphere. There are, however, over 200 species in Asia, one of which extends into Europe; about 75 species in Africa; about 200 species in Australia; and about 60 species in Oceanica. Nineteen of the genera are confined to

[1] Proc. Amer. Phil. Soc. 53:129–250. 1914.

America, and these include the only monotypic genera in the subfamily (*Orthostemon* Berg, *Psidiopsis* Berg, and *Paivaea* Berg), as well as the large and greatly differentiated genera like *Myrcia* DC. with about 450 existing species. The two other large genera, *Myrtus* Linn. with about 178 existing species, and *Eugenia* Linn. with about 1300 existing species, are the only two genera found on all the continents (except Europe), and in these two genera America furnishes 135 species of *Myrtus* and about 850 species of *Eugenia*, or over 75 per cent in *Myrtus* and over 65 per cent in *Eugenia*.

The second subfamily, the Leptospermoideae, comprises the Leptospermae with 28 genera and about 700 existing species, and the Chamaelaucieae with 12 genera and about 165 existing species. Both of these tribes are even more strikingly Australian than the Myrtoideae are American. The Chamaelaucieae are entirely Australian and are mainly confined to Western Australia, and in accordance with their peculiar habitat, specialized characters, and restricted range, are probably of relatively recent origin. The Leptospermae have a single monotypic genus in Chile, and the distribution of the other members of this tribe suggests the probability that the South American genus should be placed in some other alliance, since with the exception of *Metrosideros* Banks, which is represented in Africa, and the genus *Baeckea* Linn., which reaches the Asiatic mainland, all of the genera are confined to Australia or to the surrounding islands southeast of Asia.

ANDREWS,[2] in a recent paper, has presented some interesting statistics of distribution and an ingenious theory of the history of the family. He considers that the original stock was arborescent or shrubby, with entire, simple, opposite, penni-veined leaves with dots and intramarginal acrodrome veins; with the calyx lobes and petals imbricate, probably in fives; flowers regular, solitary or in cymes; stamens indefinite, numerous, free, with versatile, 2-celled anthers; ovary inferior, with two or more cells; style simple; fruit inferior, crowned with persistent limb of calyx, indehiscent, succulent or fleshy (rarely dry); albumen none; cotyledons thick and fleshy, with a short radicle.

[2] ANDREWS, E. C., The development of the natural order Myrtaceae. Proc. Linn. Soc. N.S. Wales **38**:529–568. 1913.

From the character of cretaceous climates this or some other theoretical prototype flourished in a mesophytic environment. Among modern groups the nearest approach to this theoretical stock is furnished by the Myrtoideae which are fleshy fruited, most numerous in species, and widely spread in the equatorial regions of the world, with over 75 per cent, however, confined to America. The existing Myrtaceae with capsular fruits, representing the extreme of specialization in the family are Australian, while the Chamaelaucieae, standing in an intermediate position between the two preceding groups, are almost wholly confined to Western Australia.

These are the facts of modern distribution. Their interpretation may be various. ANDREWS (*op. cit.*), from a study of the present distribution, geologic climates, and the geological history of the Australian region, concludes that the Leptospermoideae originated from the Myrteae, and that the cretaceous forms were widespread, which latter was undoubtedly the case. He is convinced also that before the separation of Australia from the Asiatic mainland fleshy fruited forms found themselves in a region of warm moist climate, but relatively poor soil, and that it was this edaphic factor that was the principal stimulus to the differentiation of the Leptospermoideae, which with the exception of the genus *Metrosideros* Banks show adaptations to poor soil and temperate or dry climates, and this exception explains the relatively wide distribution of *Metrosideros* from Asia to the Fiji Islands. The *Eucalyptus* forms, according to the view of this student, were derived from *Metrosideros* after the separation of New Caledonia from Australia and the latter continent from Asia. To support this latter point ANDREWS is obliged to consider all of the cretaceous identifications of *Eucalyptus* and all of the tertiary identifications outside of Australia as equally misleading. With regard to the presence of *Eucalyptus* in North America I think this contention to be not unlikely, for although in accordance with paleobotanical usage I have identified numerous forms of *Eucalyptus* in the North American Upper Cretaceous, I have long thought that these leaves represented ancestral forms of *Eugenia* or *Myrcia*, but have hesitated suggesting any change based merely on personal opinion and also

from a consideration that such change in nomenclature is undesirable at the present time from the standpoint of stratigraphic paleobotany, which in this country, at least, is a most useful handmaid of geology.

The supposed cretaceous fruits of *Eucalyptus* have long since been shown to represent *Dammara*-like forms, and in my studies of the tertiary floras I have refrained from referring any of the numerous and unquestionable myrtaceous leaves to the genus *Eucalyptus*. Regarding the possible occurrence of *Eucalyptus* in the Tertiary of Europe, I am not sure that all of the identifications of HEER, UNGER, ETTINGSHAUSEN, and others are erroneous. Certain remains considered as *Eucalyptus* fruits by these authors seem very convincing from the published figures, and furthermore there is not the slightest doubt that the other great Australian alliance of the existing flora, the Proteaceae, was represented in both Europe and America during the Cretaceous and the Tertiary. There is an additional argument against the cretaceous radiation and the paleobotanical determination of *Eucalyptus* which is furnished by the great persistence in the modern forms of the peculiar juvenile, opposite, cordate, sessile, and horizontal leaves, a feature which must represent an ancestral character of long standing before the evolution of the falcate leaves of the genus with twisted leafstalks and other xerophytic features.[3]

I have dwelt at some length on this question because of its phylogenetic importance and the possible bearing of the American lower eocene floras on this point. In considering the morphology of the existing species, *Eugenia* has many claims to be considered the most primitive, although *Myrcia* is almost equally old and is certainly closely related to *Eugenia*. Among the numerous cretaceous fossils from North America now referred to *Eucalyptus* there is not a single one that does not exhibit characteristic features of *Eugenia* or *Myrcia*, especially of the latter, a fact greatly impressed on me in handling a large amount of recent material during my study of the American tertiary forms.

[3] See DEANE, H., Observations on the tertiary flora of Australia. Proc. Linn. Soc. N.S. Wales. 15:463–475. 1900; and CAMBAGE, R. H., Development and distribution of the genus *Eucalyptus*. Presidential address, Jour. Proc. Roy. Soc. N.S. Wales, 1913.

In the lower eocene flora of the Mississippi embayment region there are six well marked species of *Myrcia* and four nearly equally well marked species of *Eugenia*, as well as a single species of *Calyptranthes*. The latter genus appears also to be represented in recent collections from the Oligocene of the Isthmus of Panama. Without pursuing the subject beyond the known facts, confessedly meager, and noting the presence in our lower eocene flora of numerous Combretaceae based upon leaves, flowers, and fruits, and a representative of the great tropical family Melastomaceae, largely American in the existing flora, both of which are families closely related morphologically to the Myrtaceae, it would seem that the known facts, as well as the law of probabilities, suggest America as the original home of the family. That in its early deployment it reached Europe, either by way of Asia or the North Atlantic plateau, early in the Upper Cretaceous, and became cosmopolitan before the close of the Cretaceous seems a most probable hypothesis. During the late Tertiary this ancestral stock, which largely coincided with the existing subfamily Myrtoideae, was forced to withdraw from temperate North America to the American tropics, where it had originated and to which it has since been so largely confined.

The types peculiar to the Australian region represent the relics of the cretaceous radiation with numerous new types evolved on that continent in the manner that ANDREWS has suggested, and at a comparatively recent date geologically. This is exactly the reverse of the hypothesis proposed by DEANE (*op. cit.*), but one that accords far better not only with the facts of geologic history, but also with those of existing distribution.

All of the American lower eocene forms are coastal types closely related to existing American species of similar habitat.

About 150 fossil forms have been referred to the family Myrtaceae, one-third at least having been described as species of *Eucalyptus*. At least half of these occur in the Cretaceous of all parts of the world, but particularly throughout the Northern Hemisphere. They are especially well represented in North America, and the possibility that they are ancestral forms of *Myrcia* or *Eugenia* has already been pointed out. A similar widespread distribution but less specific variation characterizes the

eocene forms that have been referred to *Eucalyptus*. The oligocene records are all European and the miocene records include both Europe and Asia.

The genus *Myrtus* L. has about 24 fossil species, all European, the majority being almost equally divided between the Oligocene and the Miocene. The oldest forms are early eocene, but the form-genus *Myrtophyllum* Heer has several upper cretaceous species in Europe, America, and Australia, as well as tertiary species in Europe, Asia, and South America.

The genus *Myrcia* DC., so well represented in the Lower Eocene (WILCOX) of our southern states, has species in the Middle Eocene (Claiborne) of the Mississippi embayment area, in the Oligocene (Vicksburg) of Louisiana, and in the European Oligocene. There are four species in the early Tertiary (Eocene or Oligocene) of Chile, one in the Tertiary of Ecuador, and one in the Pliocene of Brazil.

The genus *Eugenia* Linn., also prominent in our lower eocene flora, has its oldest known species in the Dakota sandstone (Upper Cretaceous) of the Rocky Mountain area. It is represented in Europe throughout the Tertiary from the Lower Eocene to the Pliocene, and is recorded by ENGELHARDT from the Tertiary of Ecuador.

The genus *Myrciaria* Berg, often included in *Eugenia*, has about 60 existing species, all American, and found in the area extending from the West Indies to Brazil and Peru. It is represented, according to ENGELHARDT, in the Tertiary of Ecuador.

The genus *Callistemon* R. Brown has been identified in both the Upper Cretaceous and the Tertiary of Europe, and no less than 25 fossil species have been referred to the genus *Callistemophyllum* Ettingshausen. These include upper cretaceous forms in both America and Europe; eocene or oligocene forms in Greenland; and numerous oligocene and miocene species in Europe and Australia.

The genus *Metrosideros* Banks, with an existing species in South Africa, another in the Sunda Islands, and 18 or 20 species in Australia or Polynesia, has 8 or 10 fossil species. The oldest, probably an erroneous identification, is recorded from the Atane

beds (Upper Cretaceous) of Greenland. There are 4 species in the Oligocene of Southern Europe and 2 species in the Miocene of that continent.

Leptospermum Forster, *Leptospermites* Saporta, and *Leptospermocarpum* Menzel have been identified from the Upper Cretaceous and Tertiary of Europe. *Tristania*-like fruits have been described as *Tristanites* by SAPORTA from the Lower Miocene of France and by KITSON from the Miocene of Australia. The genus *Psidium* Linn., with about 100 existing species in the West Indies and Mexico, is represented in Chile by an early tertiary species.

It will be seen from the foregoing enumeration that the facts of recent distribution lend support to the thesis of origin which is put forward, and this conclusion is not negatived but supported to a considerable extent by the confessedly meager evidence available in the geological record as at present known.

JOHNS HOPKINS UNIVERSITY
BALTIMORE, MD.

GROWTH AND COLLOID HYDRATATION IN CACTI

Esmond R. Long

(WITH TWO FIGURES)

During the growing season of 1914 (March to August), attempts were made to correlate growth rates of cacti in solutions of varying reaction and concentration with the hydratation phenomena exhibited on placing cut pieces of the same species in similar solutions.

It was thought at first that the parallelism discovered by Borowikow[1] in the growth rate and hydratation of *Helianthus annuus* might be expressive of a general property of plant colloids. This investigator found that acidity is a favoring factor in both the growing and the swelling rate of *Helianthus*, the amount of swelling and the amount of acceleration of growth being dependent upon the concentration of the acid, and differing with different acids, and that alkalies increase swelling and to a greater extent than acids, but do not appreciably affect growth. Dachnowski[2] has obtained somewhat parallel results on the effect of acid with beans.

Experiments of a similar nature were performed in this laboratory upon *Opuntia Blakeana*, hydrochloric and malic acids (the latter being the acid existing in greatest concentration in the sap) being used as standards for the effect of acid, and sodium hydroxide as the standard for the effect of alkali. Growth rate and swelling were studied also in a nutrient solution made by diluting to 1100 cc. 100 cc. of the following solution: calcium nitrate 6 gm., potassium nitrate 1.5 gm., magnesium sulphate 1.5 gm., potassium monohydrogen phosphate 1.5 gm., sodium chloride 1.5 gm., and distilled water 600 cc.

For the experiments on growth, joints of *O. Blakeana* matured in 1913, bearing a small flower bud or new joint, were used, the old

[1] Borowikow, G. A., Biochem. Zeitschr. 48:230–246; and 50:119–128. 1913.

[2] Dachnowski, A., Amer. Jour. Bot. 1:412–439. 1914.

joint being cut off smoothly at the base and placed in the appropriate solution, the latter being replaced by freshly prepared solution every few days. All the plants were suspended by means of wooden clamps in the various solutions to a uniform depth of 5 cm., and the battery jars containing the preparations were exposed to full sunlight in the laboratory court. While temperature and light conditions were thus not controlled, they were the same for all of the plants. The length of the new joint or bud was then measured at intervals of 3 or 4 days. In the swelling experiments, sections of standard size from healthy plants were cut with a cork borer, weighed, immersed in the different media, and kept in the dark in the laboratory constant-temperature room, the imbibed water being determined by weighing at 6-hour intervals during the following 24 hours. Inasmuch as there is considerable individual variation in swelling power in different joints from the same species, in all cases the experiments were conducted in such a way that all the joints used had equal representation in all the solutions of that experiment.

It developed immediately that for *Opuntia Blakeana*, with the concentrations of acid which it was practical to use with this plant, which has a concentration varying between N/10 and N/20 in its own sap, the observations recorded above for *Helianthus* do not hold. The results of these experiments are summed up in figs. 1 and 2 and table I, growth increment being expressed in millimeters,

TABLE I

GROWTH OF FLOWER BUDS OF *Opuntia Blakeana*, MARCH 25–APRIL 24

Medium	Total growth increment before flowering	Time in days
Distilled water	42.0 mm.	27
N/50 NaOH	40.5	30
N/50 malic acid	36.0	28
N/50 HCl	31.0	28

and swelling in percentage of the original weight. In nearly all cases the recorded figures represent the average for a number of plants, all of the hydratation experiments being carried out at least four times, and the majority of them twelve times.

The most striking fact brought out by the experiments is the inhibiting effect of acids upon both growth and hydratation, quite the opposite result from that obtained by BOROWIKOW with *Helianthus*. The discrepancy may be due in part to the stronger concentrations used in the experiment with *Opuntia Blakeana*,

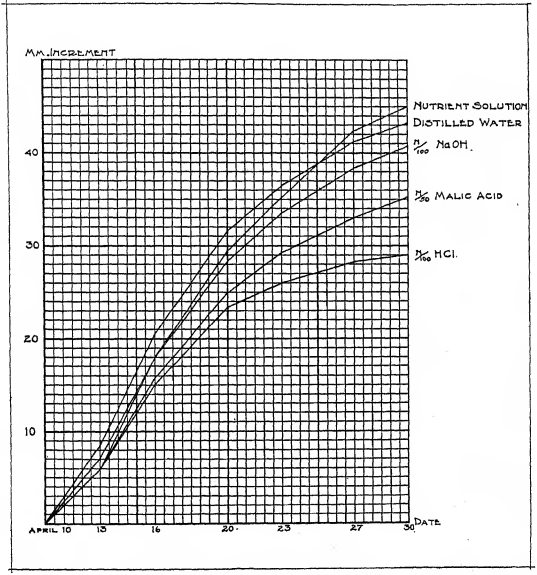

FIG. 1.—Growth of new joints of *Opuntia Blakeana*

BOROWIKOW's concentrations ranging from N/100 to much lower. It might be argued that N/50 or even N/100 HCl could be toxic in other ways than in their effect upon water imbibition. The same could hardly be held, however, concerning N/50 malic acid, as this is considerably below the concentration of the same acid

in the cell sap. Nor is it likely that the lower swelling in acid solution is to be accounted for on the ground of osmotic pressure difference. The data in table II will give some idea of the rôle of osmotic pressure in water imbibition in this plant. The figures

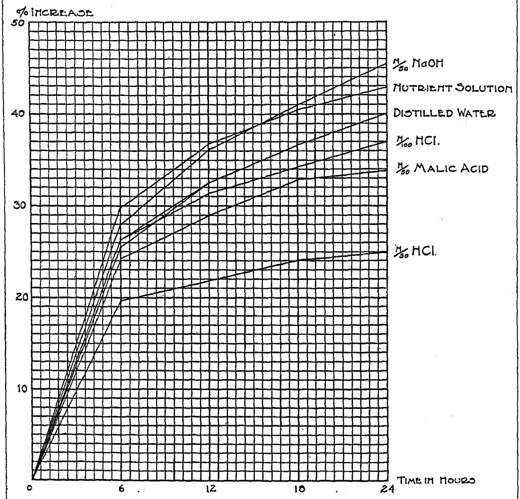

FIG. 2.—Hydratation of *Opuntia Blakeana*

given are for four pieces of *Opuntia Blakeana* in each solution, similar to those used in the hydratation experiments.

As may be seen, the swelling in distilled water and N/50 KNO_3 is practically the same, the slightly higher osmotic pressure of the latter being without measurable effect as regards increase in weight

due to imbibed water. It may be assumed that the osmotic pressure of the less ionized malic acid in N/50 concentration would also be without appreciable effect in the experiments in question.

TABLE II

Solution	Wt. at start	Wt. after 24 hrs.	Percentage increase
Distilled water................	17.64 gm.	22.59 gm.	28.1
N/50 KNO_3..................	17.83	22.88	28.3
N/10 KNO_3..................	15.65	19.40	23.9
N KNO_3..................	16.75	13.77	−17.8

It seems probable, in view of these facts, that the lowered swelling in acid solution is the direct effect of the acid ion upon the colloids which take part in the swelling. The slower *growth* rate in acid solutions is significant in this connection.

The action of alkalies upon swelling was not so regular in its effect as that of acids. While a swelling of lower degree than that produced in distilled water was practically invariable with acids, the swelling produced in alkalies was sometimes greater and in other cases less than that taking place in distilled water, and no satisfying explanation can be given for this behavior. The varying acidity of the plant itself was perhaps a factor, by neutralizing to a greater or less degree the alkali of the penetrating medium. The curve represents the average of all cases examined.

It is interesting to observe that in nutrient solution, practically always a slightly increased swelling over that taking place in distilled water occurred, and furthermore that this was paralleled, as might be expected, by a greater growth rate in this medium. The mechanism of this action, which took place in a neutral medium, was not determined.

In general, growth and swelling in these experiments paralleled rather closely nutrient solution exerting an accelerating effect over that observed in distilled water, and hydrochloric and malic acids an inhibitory one, while the effect of sodium hydroxide was irregular.

While no attempt was made to separate and determine the colloid constituents of the plant to the presence of which the swelling

was due, it is evident that substances of much different physical nature from such colloids as gelatin and fibrin, which show a marked increase in swelling in acid solutions, are concerned. It should be noted that common cornstarch (boiled and dried) swells no more in N/50 and N/25 HCl than in distilled water, although the effect of NaOH of the same molecular strength in this direction is marked.

One important point left to be considered is the effect of the varying acidity of the plant sap upon the water-absorbing power of the plant. Dr. MacDougal, to whom I am indebted for many suggestions in this work, has observed that the greatest growth rate of *Opuntia Blakeana* takes place in the day. In the daytime the acidity of the plant sap is on the decrease, the acid products of carbohydrate metabolism, after accumulating during the night, being destroyed by light and increased temperature during the day.[3] Can it be that this decrease has an effect upon the water-absorbing power and in consequence upon growth? The following experiment was carried out to test this. A joint of *Opuntia Blakeana* was cut at 5:00 A.M., 0.75 hr. before sunrise, the period of maximum acidity. Four disks were cut from it, and the holes plugged to prevent abnormal evaporation. The swelling of these in one hour in distilled water at 25° C. in diffuse light was determined immediately. The joint was exposed to the bright sunlight until

TABLE III

Time	Weight in grams	Increase in grams	Percentage increase
A.M.			
5:30	23.62		
6:30	25.25	1.63	6.9
P.M.			
4:30	22.25		
5:30	25.00	2.75	12.4

4:00 P.M., the period of minimum acidity, and then four disks were cut and their swelling determined in diffuse light for one hour, also at 25° C. The results are shown in table III.

[3] Spoehr, H. A., Biochem. Zeitschr. 57:95–111. 1913.

The swelling of the *less* acid sample is much the greater, and the difference is too great to be explained on the ground of increased osmotic pressure due to the day's transpiration. This result corresponds to those recorded above which had to do with pieces of the plant in acid and neutral solutions, and may be interpreted as possibly throwing light on the varying diurnal growth rate.

Desert Laboratory
Tucson, Ariz.

CURRENT LITERATURE

NOTES FOR STUDENTS

Niter spots.—The occupation and cultivation of vast areas of semi-arid lands in Utah, Wyoming, and Colorado, which has been made possible by the development and extension of irrigation systems, has brought agricultural workers in these states face to face with many new problems. Possibly none of these has been more striking than that presented by the sudden appearance in the soil of nitrate accumulations in amounts sufficient to produce complete sterility over considerable areas which had previously been normally productive. Since the areas thus affected are widely scattered and embrace some of the most valuable agricultural lands of these states, the search for the causes of the condition has occupied the attention of a number of workers, and a very considerable literature, some of which borders upon the controversial in character, has resulted.

Headden, who has been longest engaged in studies of the problem, was first to call attention to the extent and gravity of the condition, and to point out that most cases of sudden destruction of orchards, while popularly attributed to "black alkali" (sodium carbonate) from irrigation waters, were in reality due to nitrate accumulation. Contrary to Hilgard,[1] who attributed an increase in nitrates observed in certain irrigated soils of southern California to rapid transformation of organic matter previously accumulated in the soil, Headden[2,3] has advanced the theory that fixation of atmospheric nitrogen by *Azotobacter*, with concurrent bacterial transformation of the resulting nitrogenous compounds into nitrates, is responsible for these accumulations. This author has shown that orchard trees, alfalfa, or other crops in the affected areas uniformly show characteristic injury quite unlike that produced by excessive irrigation or by sodium carbonate or other alkali salts, but identical with that produced by excessive use of nitrate fertilizers. He also attributes the rather widespread deterioration in yield, sugar content, and keeping qualities of sugar beets to accumulation of nitrates in the soil.[4]

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[1] Hilgard, E. W., Soils. New York: Macmillan. 1911. pp. 68, 69.

[2] Headden, Wm. P., The fixation of nitrogen in some Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 155. pp. 10–69. 1910.

[3] ———, Nitrates in the soil; an explanation of so-called "black alkali" or "brown spots." Bull. Colorado Agric. Exper. Sta. no. 160. pp. 1–8. 1910.

[4] Headden, Wm. P., Deterioration in the quality of sugar beets due to nitrates formed in the soil. Bull. Colorado Agric. Exper. Sta. no. 183. pp. 1–184. 1912.

HEADDEN'S studies of the chemical composition of "niter spots"[5, 6, 7, 8] have shown that, in many cases, nitrates may be present in the first foot of the soil in amounts approximating 133 tons per acre-foot, while in many cases nitrate accumulation is progressing at rates varying between 2 and 22 tons annually per acre-foot. It is HEADDEN'S contention that the quantities of nitrates found bear no constant ratio to the amounts of chlorides or carbonates present or to the total water-soluble salt content, and that the high nitrate content is confined to the first few inches of the soil, and he regards these facts, together with the extent of the affected areas and their dissimilar geological relations, as strong evidence against the theory that the condition can be due to seepage from nitrate-bearing rocks with subsequent deposition from surface evaporation.

SACKETT[9, 10] has attacked the problem from the bacteriological side and has presented evidence that *Azotobacter* is present in large numbers in irrigated soils, and that fixation of nitrogen occurs at exceptionally high rates in soils from "niter spots"; while ROBBINS[11] has shown that these soils are unusually rich both in species and numbers of Cyanophyceae, which may be considered as supplying carbohydrates available as a source of energy for *Azotobacter*. SACKETT further concludes from his studies of the ammonifying and nitrifying efficiency of Colorado soils[12, 13] that ammonification and nitrification proceed in them at rates considerably in excess of those found by workers in other parts of the United States. It must be said in criticism of SACKETT'S work that, while his own ammonification cultures were in all cases continued for a period of seven days, he has compared the amounts of ammonia produced therein

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[5] HEADDEN, WM. P., The fixation of nitrogen in some Colorado soils; a further study. Bull. Colorado Agric. Exper. Sta. no. 178. pp. 1–96. 1911.

[6] ———, The fixation of nitrogen in Colorado soils; the distribution of the nitrates and their relation to the alkalies. Bull. Colorado Agric. Exper. Sta. no. 186. pp. 1–47. 1913.

[7] ———, The excessive quantities of nitrates in certain Colorado soils. Jour. Indust. and Eng. Chemistry **6**:586–590. 1914.

[8] ———, Do Azotobacter nitrify? Science N.S. **40**:379–381. 1914.

[9] SACKETT, WALTER G., Bacteriological studies of the fixation of nitrogen in certain Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 179. pp. 1–42. 1911.

[10] ———, Bakteriologische Untersuchungen über die Stickstoffbindung in gewissen Bodenarten von Colorado. Centralbl. Bakt. **34**^{2}:81–115. 1912 (German translation of previously published paper just cited).

[11] ROBBINS, W. W., Algae in some Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 184. pp. 24–36. 1912.

[12] SACKETT, WALTER G., The ammonifying efficiency of certain Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 184. pp. 1–23. 1912.

[13] ———, The nitrifying efficiency of certain Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 193. pp. 1–43. 1914.

directly with amounts found by other workers for similar cultures run for various shorter periods, as four or six days. Such results are in no wise comparable, since the initial rate of ammonification is normally low, but increases rapidly through the first six to ten days of incubation.

In the determinations of nitrifying efficiency, SACKETT employed soils obtained from various parts of the United States as checks upon soils from different portions of the state. Here the Colorado soils were collected under uniform conditions and with bacteriological precautions, while those from other states were taken by persons unfamiliar with bacteriological technique or with methods employed in soil sampling, and were subsequently shipped for long distances in widely varying types of containers. While the author deplores the failure to employ standard methods, he nevertheless draws rather sweeping conclusions as to the comparative ammonifying and nitrifying efficiencies of the two lots of soils, and asserts that the experiments demonstrate the presence in Colorado soils of a nitrifying flora distinct, either in species or in physiological efficiency, from that elsewhere found. It would seem to the reviewer that definite conclusions are not in order until concordant results have been obtained from repeated studies in which all possibility of variation in any significant factor has been eliminated. The necessity for caution is especially great here in view of the fact that the work of KELLERMAN and ALLEN[14] in Nevada, while showing the presence of large numbers of nitrifying and ammonifying bacteria, yields no indication of abnormally high rates of activity or of unusual activity of *Azotobacter;* while the more recent work of MCBETH and SMITH, to be presently discussed, is wholly confirmatory of the results obtained by KELLERMAN and ALLEN.

At the Utah Experiment Station, the problem has been attacked by STEWART and his co-workers. In the course of their very thorough studies of the production and movement of nitrates in the soils of the Greenville farm[15, 16, 17] STEWART and GREAVES reached the conclusion that bacterial action could not be responsible for the accumulation of any large quantities of nitrates at the surface of the soil, at least in irrigated areas, since nitrates are readily displaced into the deeper layers of the soil by the downward movement of irrigation water. In more than 30,000 determinations of nitric nitrogen,

[14] KELLERMAN, KARL F., and ALLEN, E. R., Bacteriological studies of the soils of the Truckee-Carson irrigation project. Bull. U.S. Dept. Agric., Bur. Pl. Industry. no. 211. pp. 1–36. 1911.

[15] STEWART, ROBERT, and GREAVES, J. E., A study of the production and movement of nitric nitrogen in an irrigated soil. Bull. Utah Agric. Exper. Sta. no. 106. pp. 67–96. 1909.

[16] ———, The movement of nitric nitrogen in soil and its relation to "nitrogen fixation." Bull. Utah Agric. Exper. Sta. no. 114. pp. 181–194. 1911.

[17] ———, The production and movement of nitric nitrogen in soil. Centralbl. Bakt. 34^2:115–147. 1912.

extending over a period of eight years, these workers never found a nitric nitrogen content exceeding 300 pounds per acre for a total depth of ten feet. STEWART has also studied the rate at which nitrification occurs in this soil,[18] with results which indicate that the conditions rarely if ever permit this process to become intense, a conclusion in entire accord with the results of McBETH and SMITH.[19]

The extensive and carefully controlled work carried on by these investigators for the past four years at the Greenville Experiment farm has shown that nitrification is practically confined in all cases to the first foot of the soil; that the application of irrigation water invariably diminishes the nitrifying power of the soil; that the water-content of non-irrigated soil, in a region having a well-distributed annual rainfall of 15.81 inches, is entirely too low throughout the summer months to permit of active nitrification; and that the nitrification process is markedly inhibited by the addition to the soil of small quantities of ammonium sulphate (170 ppm.). The scale on which these experiments were conducted, the attention given to the control of the experimental conditions, and the entire accordance of the data obtained for the four-year period, would apparently entitle the results of these authors to acceptance as conclusive.

STEWART and his associates have advanced the theory that abnormal accumulations of nitrates, wherever these may occur in the area which was submerged during later Cretaceous time, are due to transportation and deposition of leachings from the country rock, adducing proof that throughout Utah, Idaho, and Colorado, it is quite generally the case that the chlorine content of "niter spots" increases proportionately with the increase in nitrates, which they regard as conclusive evidence of a common origin of these elements. By recalculation of data presented by HEADDEN, these authors have shown that fairly definite ratios of increase between chlorine and nitrate hold for the Colorado nitrate areas, in one of which the supposed "fixation" of 621 pounds of nitrogen was accompanied by an increase of 236,883 pounds in the chlorine content of the first acre-foot! That any material activity on the part of nitrifying bacteria can occur in the presence of the amounts of chlorine shown by STEWART and GREAVES to be present in the soils studied by HEADDEN would seem impossible to the reviewer in view of the results of LIPMAN.[20] That *Azotobacter* can be responsible for an increase in soil nitrates which has been shown to occur at rates frequently exceeding 5000 pounds per acre-foot annually would seem equally impossible, since there would be required to supply the

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[18] STEWART, ROBERT, The intensity of nitrification in arid soils. Centralbl. Bakt. 36^2:477–489. 1913.

[19] McBETH, I. G., and SMITH, N. R., The influence of irrigation and crop production on soil nitrification. Centralbl. Bakt. 40^2:24–51. 1914.

[20] LIPMAN, C. B., Toxic effects of alkali salts on soils on soil bacteria. II. Nitrification. Centralbl. Bakt. 33^2:305–326. 1912.

necessary energy, under optimum conditions, at least 100 pounds of carbohydrate for every pound of nitrate formed.[21] Under no conceivable conditions could the algal flora of a soil supply any substantial portion of the 250 tons of dextrose needed for such fixation.

The most recent contribution to the subject is a study of the nitrate content of the country rock by STEWART and PETERSON.[22] While working primarily in Utah, these authors have collected and analyzed large numbers of sandstones, limestones, and shales from widely separated localities throughout Utah, Wyoming, and Colorado. These may be considered fairly representative of the country rocks occurring in the area covered by the cretaceous and tertiary seas. They find that while the Jurassic sandstones and shales are not characterized by an unusually high nitrate content, the cretaceous and tertiary sandstones everywhere contain nitrates far in excess of the quantities present in ordinary alkali-free soils, often to the amount of one to ten tons per acre-foot; while the tertiary shales have prevailingly an even higher nitrate content. Over very extensive, wholly barren areas of virgin "clay hill" soil there is present beneath the compact, impermeable surface clay a layer of ash-like material, two to six inches in thickness, bearing 0.15 to 0.20 per cent of sodium nitrate, an amount equal to 900 to 36,000 pounds per acre-foot. The authors estimate the total nitrate content of the Book Cliffs area in Utah and Colorado as being many times greater than that of the deposits of Chile, but have nowhere found concentrations of such extent and character as would permit them to be profitably worked, a situation resembling that found by FREE[23] in southern California. STEWART and PETERSON consider that the discovery that nitrate deposits are not confined to the shales, but are generally present in the country rock, and that their amounts are everywhere materially greater than has been hitherto supposed, constitutes conclusive proof that "niter spots" are accumulations resulting from leaching, and have no relation to bacterial activities in the soil. In view of the very large accumulation of evidence against the latter hypothesis and the conclusive character of the results obtained by STEWART and his co-workers, this conclusion would appear to be wholly justified.—JOSEPH S. CALDWELL.

Some temperature effects.—In discussing some of the phytogeographic effects of winter temperature, SHREVE[24] calls attention not only to the great lack of critical data, but more especially to the fundamental error, so prevalent

[21] MARSHALL, C. E., Microbiology. Philadelphia: Blakiston & Co. 1912. pp. 272–273.

[22] STEWART, ROBERT, and PETERSON, WILLIAM, The nitric nitrogen content of the country rock. Bull. Utah Agric. Exper. Sta. no. 134. pp. 420–465. 1914.

[23] FREE, E. E., Nitrate prospects in the Amargosa Valley, near Tecopa, Cal. Circular U.S. Dept. Agric., Bur. Soils. no. 73. 1912.

[24] SHREVE, F., The rôle of winter temperature in determining the distribution of plants. Amer. Jour. Bot. 1:193–202. 1914.

in the past, of considering a degree in one part of the temperature scale the equivalent of a degree at any other part of the scale, as is done in the use of the annual mean temperature or even in totaling the degrees of temperature for the growing season. Although more attention has been given to the temperature phenomena of the growing season, he believes that the temperature phases of the frost season are perhaps of equal importance, especially in determining the distributional limits of some subtropical plants. He has already shown, as noted in this journal,[25] that the temperature conditions in mountains is often complicated by cold air drainage, but it would appear that in such situations winter temperatures are effective in determining the vertical limits of many species. Observations show that the number of consecutive hours of freezing temperature is the factor most closely corresponding in its distribution with the limitation of the species concerned. This would harmonize with SHREVE's[26] experiments with the giant cactus, which show that the number of hours of exposure to temperature below freezing determines its death, without regard (within certain limits) to the absolute minimum reached. Thus *Cereus giganteus* is unable to resist freezing of over 19–22 hours duration, while other related Arizona species withstood periods up to 66 hours, and *Opuntia missouriensis* has been known to survive 375 consecutive hours of freezing temperature in Montana. The importance is thus emphasized of applying the exact quantitative methods of physiological work to plant geography in order to place its generalizations upon a secure logical basis.

In this connection it is interesting to note the method described by McDOUGAL[27] of applying to the summation of temperature in hour-degree units for a given time a factor expressing the rate of growth of a particular species, in order to give the relative values of such temperature exposures.—GEO. D. FULLER.

Production of alcohol by higher plants.—MINENKOW,[28] investigating the question of alcohol production by higher plants fully aerated, and the influence of osmotic pressure and temperature on the process, finds that well aerated, sterile solutions of glucose (15.8 per cent), sodium sulphate (6–7.8 per cent), and di-potassium hydrogen phosphate (7.25 per cent) retard germination of *Vicia Faba* and favor alcoholic production so that the ratio of carbon dioxide to alcohol approached nearer the value observed for alcoholic fermentation than with seeds germinating in water. Growth was retarded by these

[25] BOT. GAZ. 55:263. 1913.

[26] SHREVE, F., The influence of low temperature on the distribution of the giant cactus. Plant World 14:136–146. 1911.

[27] McDOUGAL, D. T., The auxo-thermal integration of climatic complexes. Amer. Jour. Bot. 1:186–193. 1914.

[28] MINENKOW, A. R., Die alkoholische Gärung höherer Pflanzen. Biochem. Zeitschr. 66:467–485. 1914.

solutions. With increasing concentrations of glucose (4–14 per cent), mannite (4–14 per cent), and Hellriegel's nutrient solution, there is a corresponding retardation of growth and increase of alcohol production. Low or high temperatures which retard growth also favor alcohol production, while at intermediate temperatures favoring growth alcohol production is decreased. The author therefore concludes that alcohol is produced by higher plants even under conditions of complete aeration, and correlates alcohol production with retardation of growth. It is immaterial whether the retardation of growth is brought about by unfavorable temperatures, high osmotic pressures, or other factors.—H. HASSELBRING.

Vegetation about Tucson, Arizona.—SHREVE[29] has compiled an excellent brief but comprehensive guide to the features of ecological interest in the vicinity of Tucson, Arizona. In addition to the better known desert and semidesert areas immediately surrounding the city, he has included the more diversified conditions found in the adjacent Santa Catalina mountains. Starting with a desert formation at 900 meters, in which *Cereus giganteus*, *Opuntia* spp., *Echinocactus*, and *Fouquieria splendens* are conspicuous, the desert forms are found to disappear with increasing altitude, grasses and shrubs becoming more abundant, until at 1550 meters upon the north-facing slopes there is an open forest of such species as *Juniperus pachyphloea*, *Quercus oblongifolia*, *Q. Emoryi*, *Arctostaphylos pungens*, *Rhus trilobata*, and other woody forms. A further ascent of some 500 meters reveals forests of *Pinus arizonica* and smaller stands of other pines and oaks, with specimens of *Arbutus arizonica*. Finally, at 2350 meters this interesting succession finds its climax upon slopes forested with *Pseudotsuga*, *Abies concolor*, and *Pinus strobiformis*, with even more mesophytic forms along the water courses and in the undergrowth. A brief analysis is also presented of the factors which cause this diversity of vegetation. —GEO. D. FULLER.

Water reaction in a liverwort.—CANNON[30] reports experiments with a species of *Plagiochasma* found upon arid slopes of the Santa Catalina mountains, Arizona, at an altitude of 5000 feet, showing that the thalli are able to become air dry, involving the loss of over 70 per cent of their original weight; but upon being given water again they continued to grow without apparent injury. He has also demonstrated that these plants may endure such a desiccated condition for at least 25 days, and upon their restoration to moist conditions at once assume active growth. These experiments show clearly that this liverwort can withstand in nature conditions of extreme aridity.—GEO. D. FULLER.

[29] SHREVE, FORREST, A guide to the salient physical and vegetational features of the vicinity of Tucson, Ariz. International Phytogeographic Excursion in Amer. pp. 11. 1913.

[30] CANNON, W. A., A note on the reversibility of the water reaction of a desert liverwort. Plant World 17:261–265. 1914.

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Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold face** type; synonyms in *italic.*

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